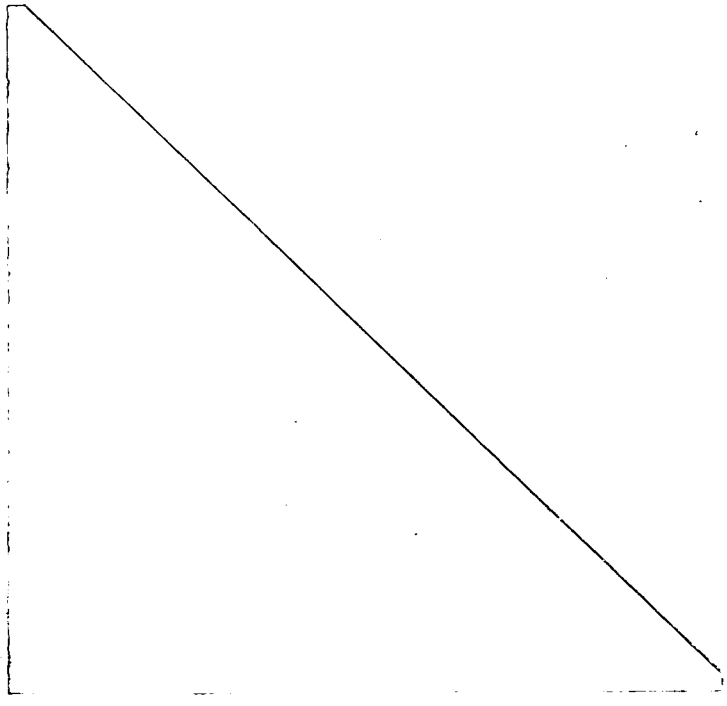
PLANT CELL BIOLOGY AND DEVELOPMENT 12



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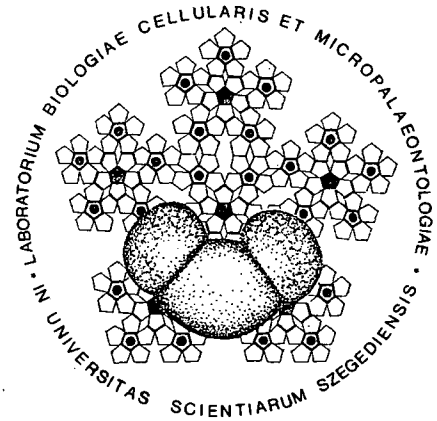

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Preface

The activity of our Laboratory at the end of the last Century and the results of the intensive international joint research programs increased the scientific production. This is the reason that another number of Plant Cell Biology and Development will appear this year. As it was emphasized in the previous, jubilee number this year is an important date in Hungary, for the Hungarian Academy of Sciences, and for our Laboratory. The 10th Anniversary of the Laboratory will be held 21th August, 2000. After the 5th Anniversary each year there was an exclusive reception in the Laboratory. At this occasion the honours of the Laboratory were awarded. Till this time a Diploma and the Commemorative Medal were the awards of our Laboratory. For the really very important date another medal was founded, the Millennium Medal of the Laboratory. Two silver and 14 copper alloy medals will be distributed only. The Chronicle of the number 13, the first number of the new Millennium will summarize the most important events of the Laboratory including the holiday.

Regarding the Editorial Board there is one change. Dr. Ann CADMAN for two years left the University of Johannesburg, and for this period Dr. M. BAMFORD will replace. Another change is that the higher educational institutions of Szeged have been unifited, so the new name of the University is University of Szeged. The position and the name of the Laboratory is unchanged in contrast to some untruthful informations distributed by E-mail by some persons.

Concerning the cover of Plant Cell Biology and Development, the decision of the staff of the Laboratory was the following: Guarding the traditional format with such changes which are distinctive between the numbers of the last and the new Millennium. This number first represents this new format.

For the financial support in the publication of this number I would like to say thanks to Grant OTKA T/9 02308, and AKP 98/26.2,5/23.

Finally, many thanks to Prof. Dr. C. ALVAREZ RAMIS catedrático for her kindness, writing appreciating comments to the 10th Anniversary of our Laboratory. During the last times there is a very intensive connection between the Department of Paleontology of the Universidade Complutense de Madrid and the Cell Biological and Evolutionary Micropaleontological Laboratory.

Szeged, 1. June, 2000.

M. KEDVES
Head of the Laboratory

COMENTARIOS ACERCA DE LAS INVESTIGACIONES CONJUNTAS ENTRE EL LABORATORIO DE PALEOBOTÁNICA DEL DEPARTAMENTO DE PALEONTOLOGÍA (UCM) / UEI DE PALEONTOLOGÍA DEL INSTITUTO DE GEOLOGÍA ECONÓMICA (CSIC) DE MADRID Y EL CELL BIOLOGICAL AND EVOLUTIONARY MICROPALEONTOLOGICAL LABORATORY DE SZEGED

Mi conocimiento con el Profesor Dr. Miklós KEDVES data de 1986. El encuentro tuvo lugar en el marco de la celebración, en Salamanca del VI Simposio de Palinología en el que presentó una comunicación: – "Dégradation experimentale de la paroi pollinique" –

Nuestra participación al Congreso, – aparición de microconos conteniendo "in situ" formas polínicas, – le interesó vivamente y a través de las conversaciones mantenidas durante el Simposio coincidimos en la forma y aspectos que creíamos debían seguirse en Paleobotánica para conseguir resultados fiables.

Desde ese año intercambiamos nuestras publicaciones sin interrupción y le cursamos varias invitaciones con el fin de que visitara los yacimientos cretácicos del Borde Sur de la Sierra de Guadarrama. Estos intercambios fueron correspondidos por la Universidad József Attila de Szeged.

En 1989 fué invitado a participar en la II Conferencia Europea de Paleobotánica que organizamos en el Departamento de Paleontología en la Universidad Complutense de Madrid. El Dr. KEDVES abrió el evento con la conferencia "New trends in Micropaleontological researches". Durante posteriores estancias en Madrid dictó otras tres conferencias.

La Academia de Ciencias de Hungría y el Consejo Superior de Investigaciones Científicas nos concedieron proyectos de investigación conjuntos y numerosas estancias en el marco de los Acuerdos bilaterales entre España y Hungría.

Como fruto de nuestra colaboración hemos publicado 6 trabajos en revistas españolas y húngaras.

En el IX Simposio de Palinología (APLE) celebrado en las Palmas de Gran Canaria en 1992 así como en el XI Simposio del APLE que tuvo lugar en Alcalá de Henares en 1996 presentamos los resultados de nuestras comunes investigaciones.

Todos los colaboradores españoles que hemos tenido la satisfacción de participar en estas investigaciones, especialmente Mª Teresa FERNÁNDEZ MARRÓN, Pilar CLEMENTE BELMONTE, Paloma GÓMEZ PORTER y la firmante de esta nota felicitamos al Prof. KEDVES en el décimo aniversario de la creacion del Laboratório de Biologia celular y Micropaleontología evolutiva, de la Universidad de József Attila de Szeged.

Concepción ALVAREZ RAMIS

1. LM AND TEM INVESTIGATIONS ON THE UPPER CRETACEOUS AJKAITE, OF HUNGARY I.

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Abstract

The fossil resin, (amber) occurring in the brown coal basin of Ajka in Hungary was denominated as Ajkaite. Multidisciplinary researches were started in our Laboratory on this kind of amber with the following purposes: 1. LM investigations on the plant microfossils (spores, pollen grains, tissue remnants) of the amber containing brown coal samples and other sedimentary types of the brown coal basin of Ajka. 2. Transmission electron microscopy of the Ajkaite to investigate the ultrastructure of the embedded organic material. 3. The aim of the first rank is to discover spores and pollen grains with well preserved intine and protoplasm in the Ajkaite and investigate the ultrastructure of the fossil protoplasm. In this first paper some previous palynological data of the investigated amber embedding coal sample, and the first TEM results on the organic material of some Ajkaite samples are presented.

Key words: Ajkaite, LM and TEM study, Upper Cretaceous, Hungary.

Introduction

The investigation of the chemofossils of amber to establish the "amber tree" was the subject of several papers. *Gymnosperm* origin: Possibly *Taxodioxylon* sp., HEY (1962), *Cryptomeria (Taxodiaceae), Cupressaceae,* HEGNAUER (1962), *Agathis,* THOMAS (1969), *Araucariaceae*, GOUGH and MILLS (1972). *Angiosperm* origin: *Hymenaea, Fabales* LANGENHEIM (1966), *Pistacia*, LANGENHEIM (1969), *Burseraceae*, VÁVRA (1991), KOSMOWSKA-CERANOWICZ, KRUMBIEGEL and VÁVRA (1993).

The well preserved animal remnants in the amber were well known a long time ago. WALTHER (1911) summarized that about 20.000 fly, and 13.500 further different kinds of arthropodous specimens were previously described. GYŐRFFY (1929) summarized the fossil moss taxa which were determined from amber samples of different ages. Among recent monographs those of POINAR Jr. (1992), was reviewed by WHALLEY (1992). First TEM results on the soft tissue of fossil fly (*Mycetophilidae: Diptera*) were published by POINAR Jr. and HESS (1982). Nucleus, cytoplasmatic vacuoles, cytomembranes, mitochondria, endoplasmic reticulum, plasma membrane were discovered.

From palynological point of view extremely important results were presented by DEJAX (DE FRANCESCHI, DEJAX and PLOËG, 1999); at the XVIth A.P.L.F. Symposium. Pollen grains were investigated with the LM and SEM method isolated from the amber

of Sparnatian sediments of the Paris Basin. Based on the presented LM data the intine and the protoplasm were in excellent preservation. This called our attention for the combined investigations on the fossil resin of Ajka.

The aim of our investigation is the following:

1. To investigate the full plant microfossil assemblages (spores, pollen grains, plant tissue remnants) of the Ajkaite containing brown coal in taxonomical, taphonomical and in paleoecological point of view.

2. To start to elaborate methods for the transmission electronmicroscopical investigations of the Ajkaite together with the embedded organic material.

3. Combined investigation of the ultrastructure of several Ajkaite samples, in the hope that well preserved protoplasm will also be found in the fossil spores and pollen grains. In this respect the early *Brevaxonate* pollen grains (*Normapolles*) seem to be the most importants for our researches.

Previous results on the amber containing brown coal layers of Ajka

HANTKEN (1867) published first that the brown coal of Ajka contains amber. SZABÓ (1871) established that the resinous remnants increase the caloric value of the coal. HLASIWETZ (1871) analyzed first the chemical compounds of the Ajkaite.

ZECHMEISTER (1926) investigated the origin of Ajkaite and he classed into the succinite and retinite. ROZLOZSNIK (1940) believed that the Ajkaite based on the chemical composition may be classed between the resinite and trinerite. SZÁDECZKY-KARDOSS (1953) observed a process of the liptobiolith and he concluded that the coal of Ajka originates of karstic swamp wood. The acidic water of the karstic swamps was neutralized or alkalined by the limestone and the microorganisms became very numerous. The sulphur content of the proteins of the microorganisms accumulate in the brown coal. PAÁL (1961) pointed that in the V. layer of the Kossuth pit the amber is common, but the microspores are sporadic. As regards the origin of the Ajkaite PAÁL (1961) based on an unpublished paper of TASNÁDI-KUBACSKA and GÓCZÁN emphasized that these may be the product of tropical decidous woods similar to kopal. In the same year, 1961 GÓCZÁN published the following; p. 796: "Das Ajkait ist wahrscheinlich ein fossiles Harz einer Kopal-Art."

GREGUSS (1949) described as *Podocarpoxylon ajkaense* n. sp. a fusit remnant from the coal basin of Ajka. The fossil material was compared with the recent *Podocarpus neriifolius*, and an oceanic clima was reconstructed. Palynological researches were carried out by GÓCZÁN (1964), GÓCZÁN, GROOT, KRUTZSCH and PACLTOVÁ (1967), KOHN (1969), etc.

Materials and Methods

Two kinds of material are the subject of our first investigations: 1. Collection of Ass. Prof. M. SZÓNOKY (Department of Geology and Paleontology of the J.A. University, Szeged), 2. Samples collected by G. KOVÁCS PhD Student from the spoil-bank of the Ajka mine. The brown coal samples were treated with the HNO_3, KOH, and HF method, the slides were mounted in glycerine-jelly hydrated of 39.6 %. For transmission electronmicroscopical investigations small pieces of Ajkaite were placed into the gelatine capsules and some drops of propylenoxyde were added to dissolve the surface of the Ajkaite. We used partial and complete dissolution of the Ajkaite, some samples were

embedded in Araldite (Durcupan, Fluka) without previous propylenoxyde treatment. The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome in the Electron Microscope Laboratory of the Department of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences. The TEM pictures were taken on a Tesla BS-540 instrument (resolution 6-7 Å).

Results

Spores and pollen grains isolated from the Ajkaite containing brown coal

Spores

Appendicisporites cf. *tricuspidatus* WEYLAND et GREIFELD 1953 (Plate 1.1., fig. 1), *Punctatisporites aquisgranensis* WEYLAND et KRIEGER 1953 (Plate 1.1., figs. 2,3).

Pollen grains

Gymnospermatophyta

Araucariacites australis COOKSON 1947, *Araucariaceae* (Plate 1.1., fig. 4)

Angiospermatophyta

Brevaxones

Normapolles

Hungaropollis ajkanus GÓCZÁN 1964 (Plate 1.1., figs. 5-8), *H. krutzschi* GÓCZÁN 1964 (Plate 1.1., figs. 9-10), *Hofkeripollenites hemimechanicus* (PFLUG 1953a) KEDVES et HERNGREEN 1980 (Plate 1.1., figs. 11,12), *Trudopollis articulus* WEYLAND et KRIEGER 1953 (Plate 1.1., figs. 13,14), *Minorpollis gallicus* KEDVES 1969 (Plate 1.1., figs. 15,16), *Semioculopollis* cf. *maestrichtiensis* KEDVES et HERNGREEN 1980 subfsp. *maestrichtiensis* (Plate 1.1., figs. 17,18), *S. granulosus* KEDVES et HERNGREEN 1980 (Plate 1.1., figs. 19,20), *S. croxtonae* KEDVES 1980 (Plate 1.1., figs. 21,22), *Oculopollis minoris* KRUTZSCH 1973 (Plate 1.1., figs. 23,24), *O. orbicularis* GÓCZÁN 1964 (Plate 1.1., figs. 25-28).

In the slides some *gymnosperm* secondary woody remnant was also observed (Plate 1.1., figs. 29,30). The areolate pitting is of modern type, which also occur in the tropical *Podocarpaceae* family. The *Oculopollis* fsp. containing amber piece (Plate 1.1., fig. 31) is important in the forthcoming researches.

Transmission electron microscopy of some Ajkaite samples

During our TEM investigations we have observed different kinds of organic remnants. The MAT system (Matière Amorphe en Transmission) which was introduced by LUGARDON, RAYNAUD and HUSSON (1991) was followed. But further TEM data from kerogene samples were also used.

Sample 99-KG-1 (Plate 1.2., figs. 1,2)

The organic material consists of two kinds of substances of different electron density. There are holes of different size in the amber embedded organic matter. The relatively small electron dense particles are mostly globular occasionally elongated or amorphous. At the globular and elongated form bacterial origin may be presumed.

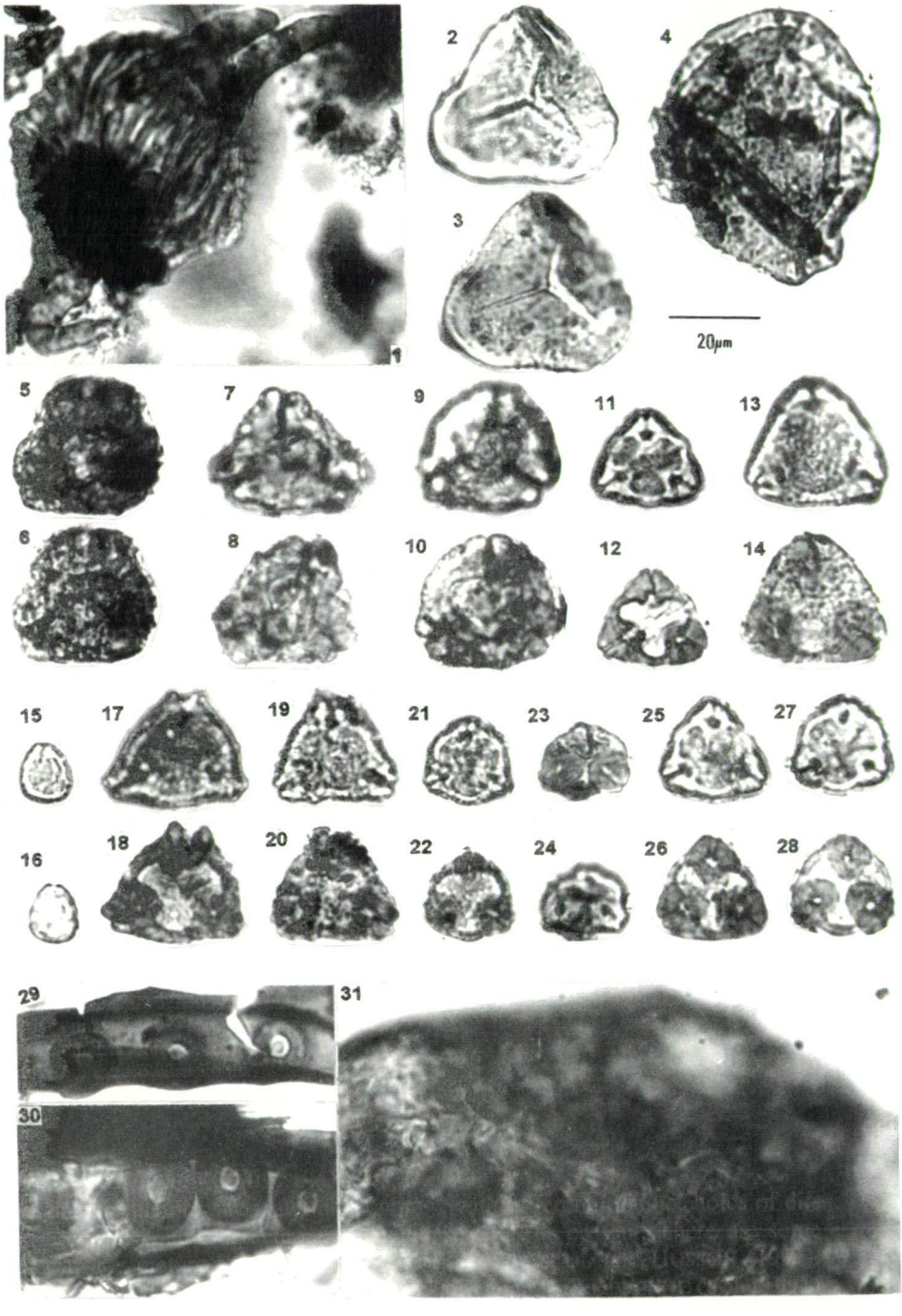
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Plate 1.1.

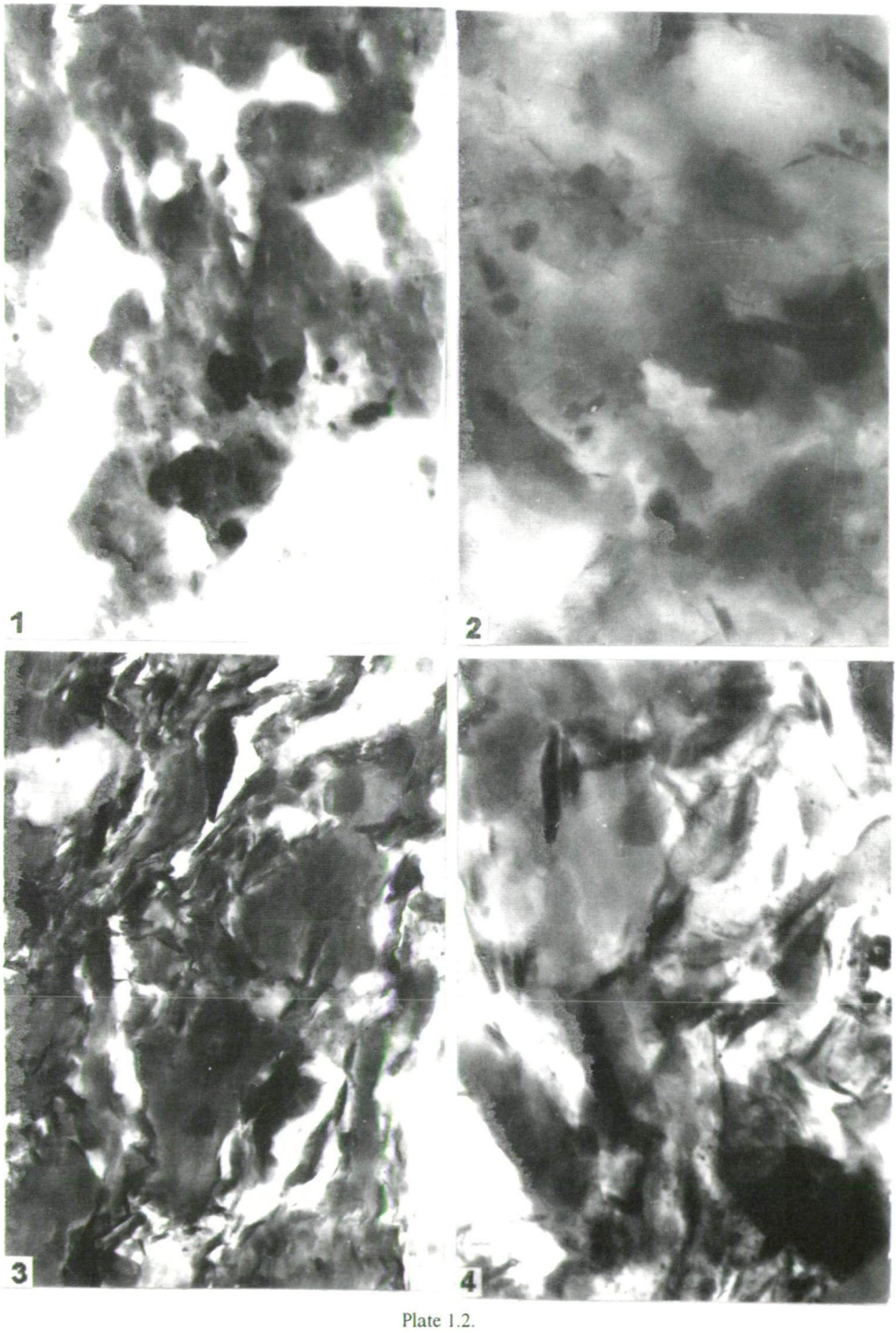

Plate 1.2.

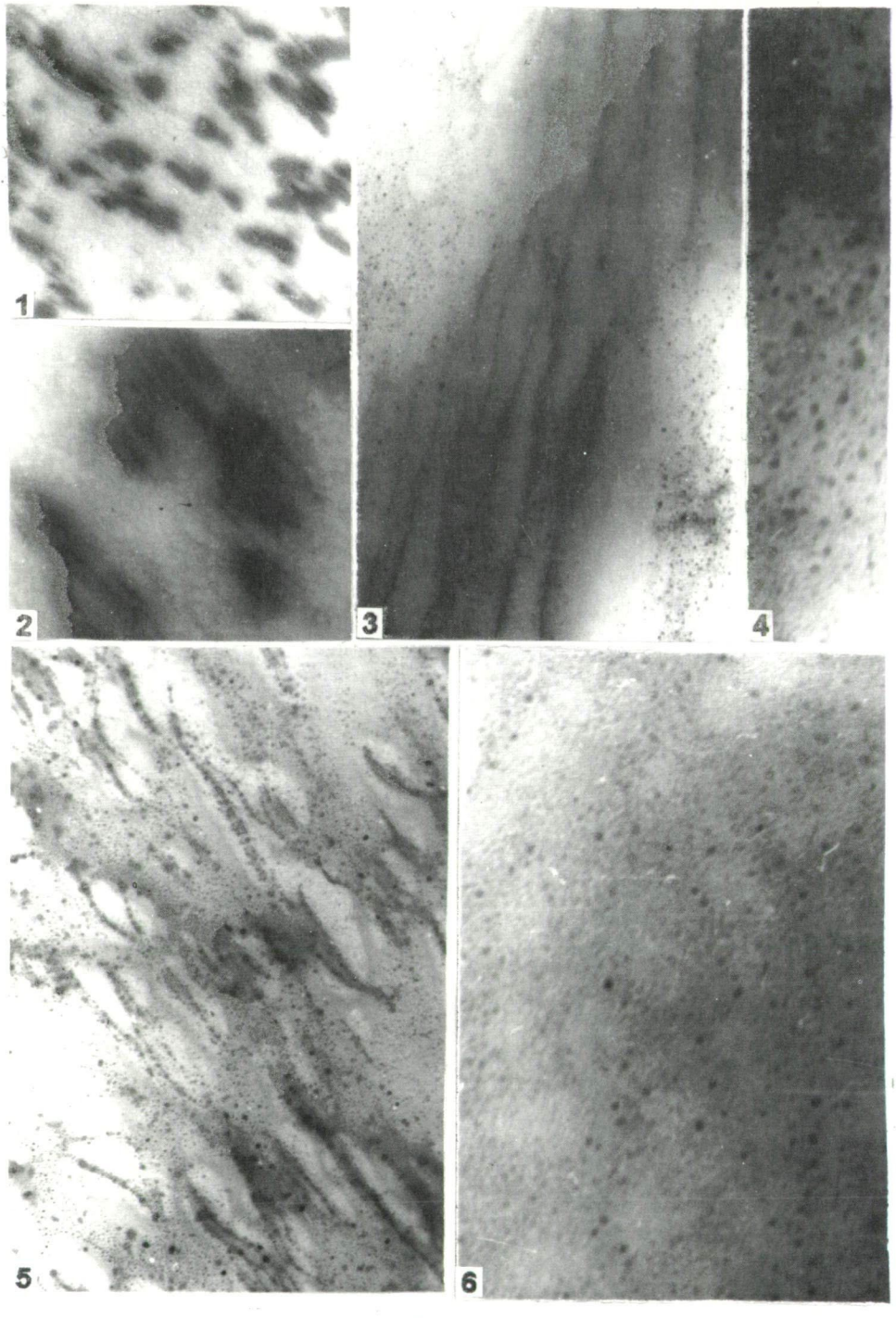

Plate 1.3.

Plate 1.1.

1. *Appendicisporites* cf. *tricuspidatus* WEYLAND et GREIFELD 1953, slide: KG-99-5; cross-table number: 19.6/126.8
2,3. *Punctatisporites aquisgranensis* WEYLAND et KRIEGER 1953, slide: KG-99-5; cross-table number: 18.2/128.4.
4. *Araucariacites australis* COOKSON 1947, *Araucariaceae*, slide: KG-99-2; cross-table number: 20.6/133.7.
5,6. *Hungaropollis ajkanus* GÓCZÁN 1964, slide: KG-99-3; cross-table number: 18.5/137.2.
7,8. *Hungaropollis ajkanus* GÓCZÁN 1964, slide: KG-99-3; cross-table number: 23.6/130.9.
9,10. *Hungaropollis krutzschi* GÓCZÁN 1964, slide: KG-99-2; cross-table number: 9.3/129.3.
11,12. *Hofkeripollenites hemimechanicus* (PFLUG 1953a) KEDVES et HERNGREEN 1980, slide: KG-99-4; cross-table number: 12.3/125.4.
13,14. *Trudopollis articulus* WEYLAND et KRIEGER 1953, slide: KG-99-4; cross-table number: 20.7/140.9.
15,16. *Minorpollis gallicus* KEDVES 1969, slide: KG-99-4; cross-table number: 19.2/128.1.
17,18. *Semioculopollis* cf. *maestrichtiensis* KEDVES et HERNGREEN 1980, slide: KG-99-5; cross-table number: 11.9/135.2.
19,20. *Semioculopollis granulosus* KEDVES et HERNGREEN 1980, slide: KG-99-5; cross-table number: 23.6/137.6.
21,22. *Semioculopollis croxtonae* KEDVES 1980, slide: KG-99-4; cross-table number: 15.2/137.2.
23,24. *Oculopollis minoris* KRUTZSCH 1973, slide: KG-99-4; cross-table number: 9.4/126.1.
25,26. *Oculopollis orbicularis* GÓCZÁN 1964, slide: KG-99-2; cross-table number: 11.6/128.4.
27,28. *Oculopollis orbicularis* GÓCZÁN 1964, slide: KG-99-3; cross-table number: 12.9/126.7.
29. *Gymnosperm* tracheid with areolate pits, slide: KG-99-4; cross-table number: 13.4/133.1.
30. *Gymnosperm* tracheid with areolate pits, slide: KG-99-2; cross-table number: 9.3/124.6.
31. *Oculopollis* fsp. pollen grains containing amber piece, slide: KG-99-2; cross-table number: 12.2/129.4.

Plate 1.2.

1,2. Ultrastructure of the MAT of the Ajkaite sample No: 99-KG-1.
1. Negative number: 7792, 15.000x.
2. Negative number: 7793, 50.000x.
3,4. Ultrastructure of the MAT of the Ajkaite sample No: 99-KG-3.
3. Negative number: 7795, 15.000x.
4. Negative number: 7796, 50.000x.

Plate 1.3.

1-6. Ultrastructure of the MAT and the Ajkaite of the sample No: 99-KG-4.
1. Negative number: 7799, 5.000x.
2. Negative number: 7800, 15.000x.
3. Negative number: 7802, 50.000x.
4. Negative number: 7802, 15.000x.
5. Negative number: 7804, 15.000x.
6. Negative number: 7702, 15.000x.

Sample 99-KG-3 (Plate 1.2., figs. 3,4)

The substance of the organic material is spongy, with different kinds of holes of different size. Sometimes not characteristic lamellar structure is present. There are some similarities with the picture published by LUGARDON, RAYNAUD and HUSSON (1991), Pl. II., fig. 2, from the sample of Upper Cretaceous of Oman, and DERENNE et al. (1991) from the *Gloeocapsomorpha prisca* from Estonian Kukersite, K1 A and B. The electron dense particles are in general elongated.

The TEM results of this sample are the most interesting, because of the variety of the observed ultrastructures. Similar to the "small elongated bacteria-like bodies" of LUGARDON, RAYNAUD and HUSSON (1991), Pl. II., fig. 7, from the Upper Cretaceous, Oman were also observed (Plate 1.3., figs. 1,2). Characteristic strands or lamelles (Plate 1.3., fig. 3), with electron dense globular particles (Plate 1.3., fig. 4) were observed. Cf. LUGARDON, RAYNAUD and HUSSON (1991), DUBREUIL, et al. (1989) from the Darwin Coorongite (Australia) and TEMPLIER et al. (1992) from the A and B races of *Botryococcus braunii* KÜTZ., after lipid extraction. There are holes in the amber (Plate 1.3., figs. 5,6) with globular electron dense particles. Picture 5 in Plate 1.3., illustrate globular electron dense particles in linear arrangement, which may also be of bacterial origin.

Discussion and Conclusions

This paper as it was emphasized presents the first results of the new research program of our Laboratory.

We have to point that in the future large LM investigations will be carried out on the sporomorphs of the Ajkaite containing layers and the woody remnants. Based on our preliminary data the investigated material may be identical with the "Upper part of the palynological zone B" Santonian of GÓCZÁN (1964). Regarding the secondary woody remnants, till this time we have observed only fragments of *gymnosperm* tracheids. The areolate pitting of the wall is modern, similar to some of the *Podocarpaceae* (cf. *Podocarpoxylon ajkaense* GREGUSS 1949), or with some so-called modern coniferous taxa. In this way the genus of *Pinus* is also possible. But it is necessary to emphasize that till this time *Araucariaceae* secondary woody remnant was not observed. The areolate pitting of the radial wall of the tracheids is very characteristic (cf. GREGUSS, 1955, 1972) as well as the pits of the cross fields.

As regards the TEM investigations, in this moment it is the most important that the ultrathin sectioning of the amber was successful, and the organic material was suitable for TEM investigations together with the amber. A remarkable number blocks will be ultrathin sectioned and investigated with transmission electron microscope. Preparation of further material for TEM investigations is in progress. We planned to select the sporomorphs containing Ajkaite pieces under the light microscope.

Our preliminary data on the MAT of the Ajkaite are encouraging for further remarkable results.

Acknowledgements

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References

COOKSON, I.C. (1947): Plant Microfossils from the Lignites of the Kerguelen Archipelago. - B.A.N.Z. Antarct. Res. Exped. (1929-31) Rep. *A*, 2, 129-142.

DE FRANCESCHI, D., DEJAX, J. et DE PLOËG, G. (1999): Pollen inclus dans l'ambre Eocène (Sparnacien) du Bassin de Paris: Observation in situ, extraction et observation en microscopie électronique à balayage et en microscopie photonique. – XVIème Symposium de l'A.P.L.F., Liège, Belgique. Résumés des communications, 37,38.

DERENNE, S., METZGER, P., LARGEAU, C., VAN BERGEN, P.F., GATELLIER, J.P., SINNINGHE DAMASTÉ, J.S., DE LEEUW, J.W. and BERKALOFF, C. (1991): Similar morphological and chemical variations of *Gloeocapsomorpha prisca* in Ordovician sediments and cultured *Botryococcus braunii* as a response to changes in salinity. - Org. Geochem. *19*, 299-313.

DUBREUIL, C., DERENNE, S., LARGEAU, C., BERKALOFF, C. and ROUSSEAU, B. (1989): Mechanism of formation and chemical structure of Coorongite-I. Role of the resistant biopolymer and of the hydrocarbons of *Botryococcus braunii*. Ultrastructure of Coorongite and its relationship with Torbanite. - Org. Geochem. *14*, 543-553.

EDELSTEIN, M. (1937): Az ajkai szén szénkőzettani vizsgálata. Mikroskopische Untersuchung der Ajkaer Braunkohle aus der oberer Kreide. - Földt. Közl. *67*, 109-131.

GÓCZÁN, F. (1961): Die Palynologie der Senon-Bildungen des Süd-Bakony. - Ann. Inst. Geol. Publ. Hung. *49*, 789-799.

GÓCZÁN, F. (1964): Stratigraphic Palynology of the Hungarian Upper Cretaceous. - Acta Geol. *8*, 229-264.

GÓCZÁN, F., GROOT, J.J., KRUTZSCH, W. und PACLTOVÁ, B. (1967): Die Gattungen des "Stemma *Normapolles* PFLUG 1953b" (*Angiospermae*). Neubeschreibungen und Revision europäischer Formen (Oberkreide bis Eozän). - Paläont. Abh. *2*, 427-633.

GOUGH, L.J. and MILLS, J.S. (1972): The composition of Succinite (Baltic Amber). - Nature *239*, 527-528.

GREGUSS, P. (1949): Az ajkai felső-krétakorú barnakőszén fuzitzárványának meghatározása (*Podocarpoxylon ajkaense* n. sp.). - Földt. Közl. *79*, 9-12.

GREGUSS, P. (1955): Xylotomische Bestimmung der heute lebenden *Gymnospermen*. - Akadémiai Kiadó, Budapest.

GREGUSS, P. (1972): Xylotomy of the living *Conifers*. - Akadémiai Kiadó, Budapest.

GYÖRFFY, I. (1929): Borostyánkövekbe ragadt mohákról és a fossilis mohák koráról. - Debreceni Szemle *3*, 65-75.

HANTKEN, M. (1867): Az ajkai kőszénképlet geológiai viszonyai. - M.F.T. Munkálatai *3*, 98-102.

HEGNAUER, R. (1962): Chemotaxonomie der Pflanzen. Bd. 1. *Thallophyten, Bryophyten, Pteridophyten*, und *Gymnospermen*. - Chemische Reihe 14, Birkhäuser, Basel und Stuttgart.

HEY, M.H. (1962): An Index of Mineral Species and varieties arranged chemically. 2nd ed., British Museum, London 24.

HLASIWETZ, H. (1871): Harz aus der Braunkohle von Ajka im Viszprimer Comitat. - Verh. Kaiserlich-Königl. Geol. Reichsanst. *1/18*, 191-192.

KEDVES, M. (1969): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne. IV. Pollens des *Normapolles*. - Pollen et Spores *11*, 385-396.

KEDVES, M. (1980): Palynological investigations on sediments of the Lower Danian (Fish Clay, Denmark). I. - Acta Miner.-Petr. Szeged. *24*, 167-186.

KEDVES, M. and HERNGREEN, G.F.W. (1980): Palynology of the stratotype of the Maestrichtian and the Gulpen Formation, ENCI Section, Maastricht, The Netherlands. - Pollen et Spores *22*, 483-544.

KOCH, A. (1871): Felsőkréta képlet a Bakonyban . - Földt. Közl. *1*, 11-12.

KOCH, S. (1985): Magyarország ásványai. - Akadémiai Kiadó, Budapest.

KOHN, I. (1969): A Jókai bánya művelés alatt álló telepeinek spóra-pollenegyüttesei. - JATE, Növénytani Tanszék, szakdolgozat, 1-83.

KOSMOWSKA-CERANOWICZ, B., KRUMBIEGEL, G. und VÁVRA, N. (1993): Glessit, ein tertiäres Harz von *Angiospermen* der Familie *Burseraceae*. - N. Jb. Geol. Paläont. Abh. *187*, 299-324.

KRUTZSCH, W. (1973): Über einige neue Sporen und Pollenformen aus dem Maastricht Norddeutschland. - Abh. zentr. geol. Inst. *18*, 77-98.

LANGENHEIM, J.H. (1966): Botanical Source of Amber from Chiapas. - Mexico-Cienc. *24*, 201-210.

LANGENHEIM, J.H. (1969): Baltic Amber - A Palaeobiological Study. In: LYMEBORG, I. (Hrsg.): Entomograph. 1. - Klampenborg. Scandinavian Science Press.

LUGARDON, B., RAYNAUD, J.-F. et HUSSON, P. (1991): Données ultrastructurales sur la matière organique amorphe des kérogènes. - Palynosciences *1*, 69-88.

POINAR, G.O. Jr. (1992): Life in Amber. - Stanford University Press.

POINAR, G.O. Jr. and HESS, R. (1982): Ultrastructure of 40-Million-Year-Old Insect Tissue. - Science *215*, 1241-1242.

PAÁL, Á.-né (1961): Az ajkai kréta kőszéntelepek kőszénkőzettani vizsgálatának eredményei. - M.Á.F.I. Évk., *49*, 871-938.

PFLUG, H.D. (1953a): In THOMSON, P.W. und PFLUG, H.D.

ROZLOZSNIK, P. (1940): A csingervölgyi bányászat múltja, jelene és jövője. Vergangenheit, Gegenwart und Zukunft des Bergbaues in Csingervölgy. - M. Kir. Á.F.I. évi jelentése az 1933-35 évekről *3*, 1231-1245.

SZABÓ, J. (1871): Az ajkai kőszéntelep a Bakonyban. - Földt. Közl. *7*, 124-130.

SZÁDECZKY-KARDOSS, E. (1953): Barna- és feketekőszén-fajtáink a népgazdálkodás fejlesztésének szolgálatában. - M.T.A. Műszaki Tud. Oszt. Közl. *10*, 39-56.

TEMPLIER, J., LARGEAU, C., CASADEVALL, E. and BERKALOFF, C. (1992): Chemical inhibition of resistant biopolymers in outer walls of the A and B races of *Botryococcus braunii*. - Phytochemistry *31*, 4097-4104.

THOMAS, B.R. (1969): Kauri resins - modern and fossil. In: Organic geochemistry - methods and results EGLINTON, G. and MURPHY, M. (eds.). - Springer, Berlin, 599-618.

THOMSON, P.W. und PFLUG, H.D. (1953): Pollen und Sporen des mitteleuropäischen Tertiärs. - Palaeontographica *B, 94*, 1-138.

VÁVRA, N. (1984): Bernstein - die Tier- und Pflanzenwelt fossiler Harze. - Schriften des Vereines zur Verbreitung naturwiss. Kenntnisse i. Wien *122/123*, 67-96.

VÁVRA, N. (1991): Chemofossils from "Baltic Amber" - a contribution to the biochemistry of the "amber tree"? - Palaeovegetational Development in Europe, 423-427.

WALTHER, J. (1911): A föld és az élet története. - K.M. Természettud. Társ. Budapest.

WEYLAND, H. und GREIFELD, G. (1953): Über strukturbietende Blätter und pflanzliche Mikrofossilien aus den Untersenonen Tonen der Gegend von Quedlinburg. - Palaeontographica *B, 95*, 30-52.

WEYLAND, H. und KRIEGER, G. (1953): Die Sporen und Pollen der Aachener Kreide und ihre Bedeutung für die Charakterisierung des Mittleren Senons. - Palaeontographica *B, 95*, 6-29.

WHALLEY, P. (1992): Ancient residents in resin. - Nature *360*, 714.

ZECHMEISTER, L. (1926): Adatok az Ajkait, egy hazai fosszilis gyanta ismeretéhez. Zur Kenntnis des Ajkaits, eines fossilen Harzes aus Ungarn. - Mat.- és Természettud. Értesítő *43*, 332-341.

2. UPPER CRETACEOUS POLLEN GRAINS FROM EGYPT V.

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Abstract

Species of the following form-genera are presented in this paper: *Scabradiporites* VARMA et RAWAT 1963, *Oculopollis* PFLUG 1953b, *Semioculopollis* GÓCZÁN, KRUTZSCH et PACLTOVÁ 1967, *Minorpollis* KRUTZSCH 1959, *Triatriopollenites* PFLUG 1953a emend. KEDVES 1982 in KEDVES et RUSSELL, *Myrtaceidites* (COOKSON et PIKE 1954) POTONIÉ 1960, *Retitriporites* (VAN DER HAMMEN 1956) GONZÁLEZ GUZMÁN 1967, *Beaupreaidites* COOKSON 1950 ex COUPER 1953, *Guzmanipollenites* n. fgen., *Scabratriporites* VAN DER HAMMEN 1956, *Syncolporites* VAN DER HAMMEN 1954, 1956 and *Triporopollenites* PFLUG et THOMSON 1953. One n. fgen. and five n. form-species are described.

Key words: Palynology, fossil, Brevaxones, Upper Cretaceous, Egypt.

TURMA: *POROSES* (*~POROSA* NAUMOVA 1937, 1939) POTONIÉ 1960

SUBTURMA: *DIPORINES* (*~DIPORINA* NAUMOVA 1937, 1939)

Form-genus: *Scabradiporites* VARMA et RAWAT 1963
Diporate, scabrate pollen grains.

1. *Scabradiporites* fsp. A
(Plate 2.1., figs. 1,2)

Description: Amb ellipsoidal. Surface scabrate. The exine is very thin, 0.3-0.4 µm and its layers were not discernible by light microscopy. Pore diameter is 4-5 µm, with an irregular margin.
Diameter: 24 µm.
Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Abu Minquar (4-3) infrequent.

2. *Scabradiporites* fsp. B
(Plate 2.1., figs. 3,4)

Description: Elongated, relatively narrow pollen grain. Surface scabrate. The exine is 0.8-1 µm thick and the three ectexine layers are equal, T/I/F = 1/1/1. Pore diameter is 0.6 µm, and at one aperture an atrium-like structure was observed, probably a result of the preservation.

Diameter: 26 µm.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Kharga (1-28) infrequent.

SUBTURMA: *TRIPORINES* (~*TRIPORINA* NAUMOVA 1937, 1939)

INFRATURMA: *NORMAPOLLES* PFLUG 1953b

New key for the *Normapolles* taxa was published by BATTEN and CHRISTOPHER (1981).

Form-genus: *Oculopollis* PFLUG 1953b

For the occurrence of this genus in Africa, see the publications of PETROSJANTZ and TROFIMOV (1971); Upper Cretaceous, Sahara and PETROSJANTZ and TROFIMOV (1975) ?Danian - Paleocene, Sahara. The first SEM data on the oculata *Normapolles* was published by KEDVES and RADVÁNSZKI (1975), and a characteristic sculpture was demonstrated by this method. TEM data from HEGEDŰS, KEDVES and PÁRDUTZ (1971), MÉDUS (1975, 1977) and KEDVES (1990).

1. *Oculopollis pertinax* (PFLUG 1953a) PFLUG 1953b
(Plate 2.1., figs. 5,6)

Description: Amb triangular, with convex sides. The apertural area is prominent. Surface granular to finely rugulate. The inter-apertural exine is 1.8-2.2 µm thick. The infratectum is a little thicker than the tectum and the foot layer. The structure is not discernible by optical microscopy, but is probably granular. The radial diameter of the oculi is 13-15 µm. The surface is mostly finely granular. The ectoapertures are relatively long colpi, which usually reach the level of the endoaperture. There is a very narrow vestibulum. The endoapertures are pori about 1.5-2 µm in diameter.

Diameter: 32 µm.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, fm. indet.: Oweina (1) infrequent.

Form-genus: *Semioculopollis* GÓCZÁN, KRUTZSCH et PACLTOVÁ 1967

The oculi are present on only one side of these pollen grains. This characteristic feature separates it from *Oculopollis* PFLUG 1953b.

1. *Semioculopollis croxtonae* KEDVES 1979
(Plate 2.1., figs. 7,8)

Description: Amb triangular, with straight or concave sides. Surface granular or finely verrucate. The inter-apertural exine is 2-2.5 µm thick, the infratectum is thicker than the tectum and the foot layer, T/I/F = 1/2-3/1. Structure is not easily discernible by light microscopy, probably granular. The oculus is present on one side, its radial diameter is 8-12 µm. Annulus is 5-6 µm thick, the foot layer is thickened around the endopore, it is a 1.5 µm thick endotumescens.

Diameter: 25 µm.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, fm. indet.: Oweina (1) infrequent.

Form-genus: *Minorpollis* KRUTZSCH 1959

Small, triatriate pollen grains, with elongated ecto- and endoapertures.

1. *Minorpollis gallicus* KEDVES 1969
(Plate 2.1., figs. 9,10)

Description: Amb triangular, with convex sides. Surface scabrate. The exine is 0.3-0.6 µm thick, but its stratification and structure is not discernible by optical microscopy. The annulus is about 0.8 µm thick. The exoaperture is 1-2 µm in diameter.

Diameter: 12 µm.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-1) infrequent.

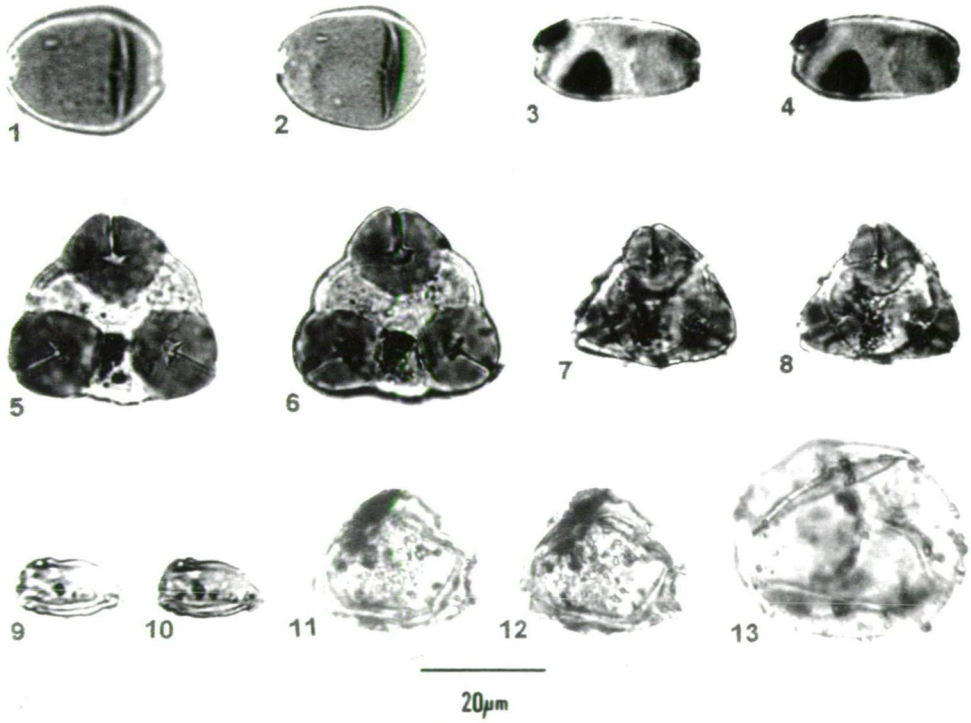

Plate 2.1.

1,2. *Scabradiporites* fsp. A, slide: Abu Minquar-4-3-5; cross-table number: 15.7/109.8.
3,4. *Scabradiporites* fsp. B, slide: Kharga-1-28-4; cross-table number: 17.6/119.9.
5,6. *Oculopollis pertinax* (PFLUG 1953a) PFLUG 1953b, slide: Oweina-1-1; cross-table number: 9.9/107.2.
7,8. *Semioculopollis croxtonae* KEDVES 1979, slide: Oweina-1-1; cross-table number: 4.8/114.8.
9,10. *Minorpollis gallicus* KEDVES 1969, slide: Farafra-6-2-1-8; cross-table number: 9.7/105.8.
11,12. *Triatriopollenites* cf. *pseudogranulatus* (GLADKOVA 1965) KEDVES 1974, *Myricaceae*, slide: 70-1-7-2-4; cross-table number: 16.4/109.5.
13. *Triatriopollenites grandis* (GLADKOVA 1965) KEDVES 1974, *Myricaceae*, slide: 70-1-7-2-6; cross-table number: 20.3/102.3.

INFRATURMA: *POSTNORMAPOLLES* PFLUG 1953b

Form-genus: *Triatriopollenites* PFLUG 1953a emend. KEDVES 1982, in KEDVES et RUSSELL

New taxonomical concepts were introduced for this triatriate pollen grains by FREDERIKSEN and CHRISTOPHER (1978) and KEDVES (1982).

1. *Triatriopollenites* cf. *pseudogranulatus* (GLADKOVA 1965) KEDVES 1974, *Myricaceae*
(Plate 1.1., figs. 11,12)

Description: Amb triangular, with convex sides. Surface punctate-granular. The inter-apertural exine is 1.5-2 µm thick. The infratectal layer is a little thicker than the tectum and the foot layer. Structure is not discernible by optical microscopy, probably granular. The annulus is 2-2.7 µm thick, the ectoaperture 2-3 µm in diameter. The atrium is narrow and long.

Diameter: 26 µm.

Remark: This is a so-called "old *Postnormapolles* type".

Occurrence and frequency in the samples investigated from Egypt: Coniacian-Santonian: Abu Rauwash (70-1-7-2) infrequent.

2. *Triatriopollenites grandis* (GLADKOVA 1965) KEDVES 1974, *Myricaceae*
(Plate 2.1., fig. 13, plate 2.2., fig. 1)

Description: Amb triangular, with convex sides. Surface punctate or finely granular. The inter-apertural exine is 0.8-1.2 µm thick. The tectum, infratectal layer, and the foot layer are of equal thickness, T/I/F = 1/1/1. The structure is not clearly discernible by optical microscopy, probably granular. The annulus is 1-1.2 µm thick and the atrium is about 2 µm wide. Diameter of the exoapertures is 2-2.5 µm.

Diameter: 37 µm.

Occurrence and frequency in the samples investigated from Egypt: Coniacian-Santonian: Abu Rauwash (70-1-7-2): infrequent.

Form-genus: *Myrtaceidites* (COOKSON et PIKE 1954) POTONIÉ 1960

Triangular, triaperturate pollen grains. Ectoapertures colpi, with arci. In the polar area there is a triangular part, which is surrounded by the arci.

1. *Myrtaceidites mesonesus* COOKSON et PIKE 1954, *Myrtaceae, Eucalyptus*
(Plate 2.2., figs. 2-5)

Description: Amb triangular, with convex sides. Surface finely granulate. The inter-apertural exine is 0.6-0.8 µm. The tectum, infratectum and the foot layer are of equal thickness, T/I/F = 1/1/1. Structure not clearly discernible by optical microscopy. Exoapertures narrow colpi, bordered by 1-2 µm wide arci. There is a tumescens-like thickening in the polar region about 2 µm in thickness. In the polar region there is a triangular area which is surrounded by arci. Endoapertures small atria.

Diameter: 13 µm; 10-15 µm.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-1) infrequent, Abu Minquar (4-3) infrequent, Kharga (1-28) common.

Form-genus: *Retitriporites* (VAN DER HAMMEN 1956) GONZÁLEZ GUZMÁN 1967

Triporate pollen grains, with reticulate sculpture. Until this time, this kind of *angiosperm* pollen grain is not so common in the Upper Cretaceous spore-pollen assemblages.

1. Cf. *Retitriporites* fsp.
(Plate 2.2., figs. 6,7)

Description: Amb triangular, with slightly convex sides. Surface reticulate. The lumina of the reticulum are 0.6-1.2 µm in size. In the apertural region there is a zone with a smooth surface about 4-5 µm in width. The inter-apertural exine is 0.4-0.5 µm thick. The exo- and endoapertures are pori, about 2-2.5 µm in diameter.
Diameter: 21 µm.
Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-1) infrequent.

ANTETURMA: *VARIGERMINATES* POTONIÉ 1970
TURMA: *PLICATES* NAUMOVA 1937, 1939
SUBTURMA: *TRIPTYCHES* NAUMOVA 1937,1939
INFRATURMA: *ISOTRICOLPATI* POTONIÉ 1970

Form-genus: *Beaupreaidites* COOKSON 1950 ex COUPER 1953 emend. MARTIN 1973

MARTIN (1973), MARTIN and HARRIS (1974), MILDENHALL (1978), and DETTMANN and JARZEN (1988, 1996) re-examined in detail the taxonomic problems of this form-genus. The pollen grains of this genus are triangular in polar view, surface sculptured, the exoapertures are short furrows.

1. *Beaupreaidites mildenhallii* n. fsp. subfsp. *mildenhallii*
(Plate 2.2., figs. 8,9)

Diagnosis: Amb triangular, with slightly convex sides. Surface reticulate. The mesh of the reticulum is 0.4-0.8 µm, and usually becomes smaller in the polar region. Muri width about 0.4 µm. The inter-apertural exine is about 1.5 µm thick. The infratectum and the foot layer is thicker than the tectum, T/I/F = 1/2/2. Structure intrabaculate. The exine becomes thinner in the apertural region and is about 0.8 µm in thickness near the apertures. The apertures are short 4-6 µm, but consists of relatively large colpi (2-4 µm).
Diameter: 27 µm; 25-32 µm.
Holotype: Plate 2.2., figs. 8,9, slide: Farafra-6-2-2-1; cross-table number: 11.6/109.4.
Locus typicus: Farafra, Maestrichtian, Nubia Sandstone.
Stratum typicum: clayey brown coal.
Derivatio nominis: In honour of Dr. D.C. MILDENHALL.
Differential diagnosis: The smaller size separates this taxon from *B. elegansiformis* COOKSON 1950. Moreover, based on the documentation of COOKSON (1950) the mesh

of the reticulum is smaller than in our new species, and the colpi of *B. elegansiformis* COOKSON 1950 are longer.

Botanical affinity: *Proteaceae.*

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-1) infrequent, Farafra (6-2-1) infrequent, Farafra (11) infrequent.

2. *Beaupreaidites mildenhallii* n. fsp. subfsp. *minor* n. subfsp.
(Plate 2.2., figs. 10,11)

Diagnosis: Amb triangular, with straight or mildly convex sides. Surface finely reticulate. The mesh of the reticulum is about 0.4 µm, with muri about 0.2-0.3 µm wide. The size of the ornamentation is the same on the different parts of the pollen grains. The exine is 2-3 µm thick and the foot layer is relatively thick, T/I/F = 1/1.5/5. Structure intrabaculate. The exine is 1.5-2 µm thick around the apertures. The furrows are short; 3-4 µm and are about 0.5 µm wide.

Diameter: 21 µm; 19-25 µm.

Subfsp. type: Plate 2.2., figs. 10,11, slide: Farafra-6-2-2-1; cross-table number: 15.3/108.9.

Locus typicus: Farafra, Maestrichtian, Nubia Sandstone.

Stratum typicum: clayey brown coal.

Derivatio nominis: From its small size.

Differential diagnosis: The smaller size separates this subfsp. from *M. mildenhallii* subfsp. *mildenhallii.*

Botanical affinity: *Proteaceae.*

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-2), infrequent, Farafra (6-2-1) infrequent.

3. Cf. *Beaupreaidites* fsp.
(Plate 2.2., figs. 12,13)

Description: Amb triangular, with convex sides. Surface reticulate. The mesh of the reticulum is 1.5-2 µm and the muri width is 0.5 µm. The exine is about 2 µm thick with the foot layer being the thickest, T/I/F = 1/1.5/3. The apertures are short furrows, 6-8 µm long and 1-2 µm wide. There are 1.5 µm exinous thickenings around the apertures.

Diameter: 19 µm.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Kharga (1-28) infrequent.

Form-genus: *Guzmanipollenites* n. fgen.

Fgen.-type: *Guzmanipollenites cretaceus* n. fsp.

(Plate 2.2., figs. 14-19)

Diagnosis: Triangular, triaperturate pollen grains. Exoapertures short furrows, endoapertures pori, with an annulus in the apertural region. Ornamentation verrucate, the sculptural elements are characteristic.

Form-genus type: Plate 2.2., figs. 14,15, slide: Farafra-6-2-2-4; cross-table number: 6.2/114.7.

Locus typicus: Farafra, Maestrichtian, Nubia Sandstone.

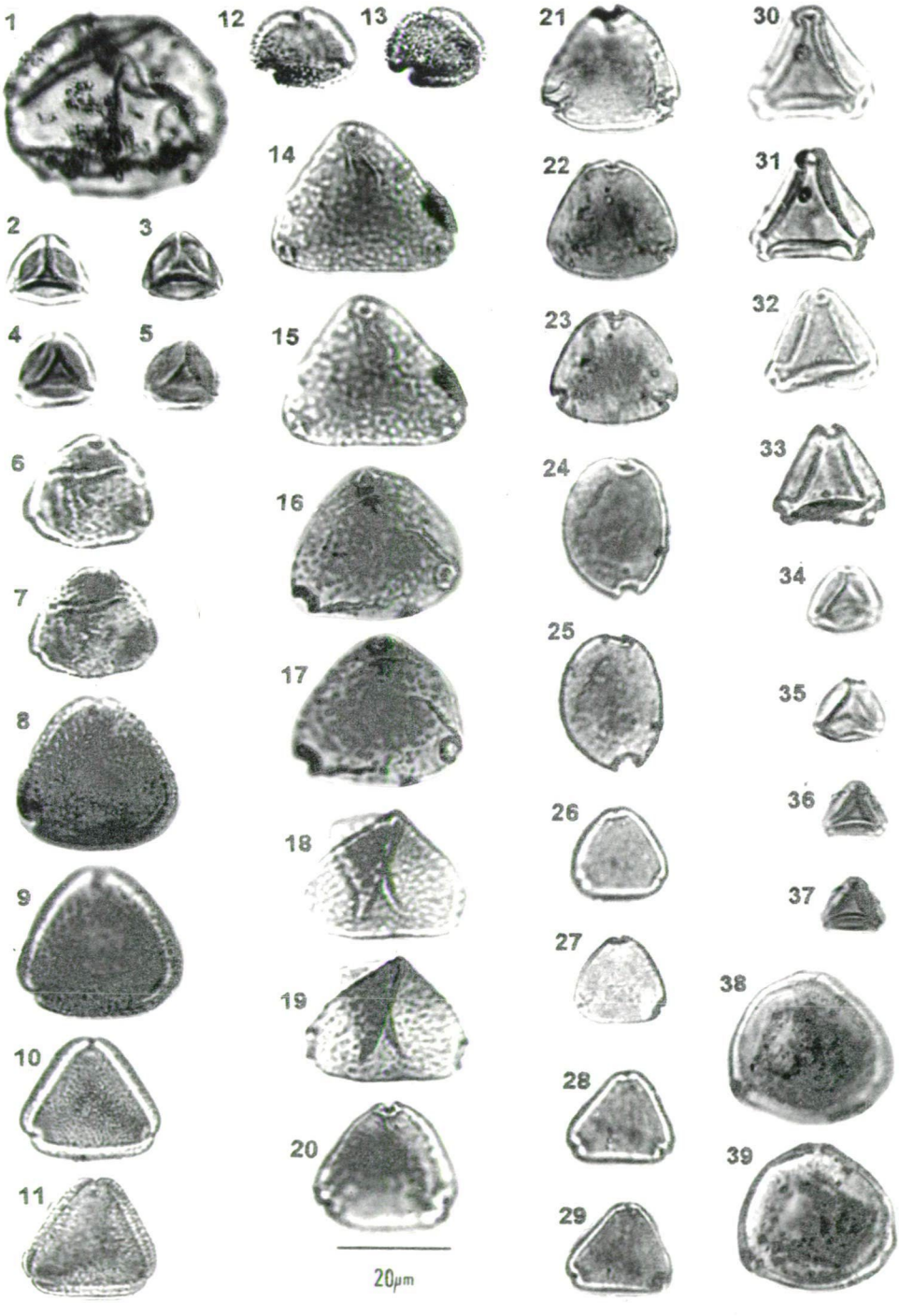
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Plate 2.2.

Plate 2.2.

1. *Triatriopollenites grandis* (GLADKOVA 1965) KEDVES 1974, *Myricaceae*, slide: 70-1-7-2-6; cross-table number: 20.3/102.3.
2,3. *Myrtaceidites mesonesus* COOKSON et PIKE 1954, *Myrtaceae, Eucalyptus*, slide: Abu Minquar-4-3-1; cross-table number: 20.4/107.8.
4,5. *Myrtaceidites mesonesus* COOKSON et PIKE 1954, *Myrtaceae, Eucalyptus*, slide: Abu Minquar-4-3-2; cross-table number: 12.6/109.8.
6,7. Cf. *Retitriporites* fsp., slide: Farafra-6-2-1-8; cross-table number: 20.1/109.9.
8,9. *Beaupreaidites mildenhallii* n. fsp.; subfsp. *mildenhallii, Proteaceae*, slide: Farafra-6-2-2-1; cross-table number. 11.6/109.4.
10,11. *Beaupreaidites mildenhallii* n. fsp. subfsp. *minor* n. subfsp., *Proteaceae*, slide: Farafra-6-2-2-1; cross-table number: 15.3/108.9.
12,13. Cf. *Beaupreaidites* fsp., slide: Kharga-1-28-3; cross-table number: 13.7/119.8.
14,15. *Guzmanipollenites cretaceus* n. fgen. et fsp., slide: Farafra-6-2-2-4; cross-table number: 6.2/114.7.
16,17. *Guzmanipollenites cretaceus* n. fgen. et fsp., slide: Farafra-6-2-2-11; cross-table number: 14.2/106.4.
18,19. *Guzmanipollenites cretaceus* n. fgen. et fsp., slide: Farafra-6-2-2-9; cross-table number: 6.1/103.1.
20,21. *Scabratriporites druggii* n. fsp., slide: Farafra-6-2-2-1; cross-table number: 20.2/109.3.
22,23. *Scabratriporites druggii* n. fsp., slide: Farafra-6-2-1-3; cross-table number: 9.8/115.8.
24,25. *Scabratriporites druggii* n. fsp., slide: Farafra-6-2-2-5; cross-table number: 14.2/103.6.
26,27. *Scabratriporites simpliformis* VAN HOEKEN-KLINKENBERG 1966, slide: Farafra-6-2-2-1; cross-table number: 16.4/103.3.
28,29. *Scabratriporites simpliformis* VAN HOEKEN-KLINKENBERG 1966, slide: Farafra-6-2-2-1; cross-table number: 6.8/107.9.
30,31. *Syncolporites jardinei* n. fsp., slide: Farafra-6-2-2-8; cross-table number: 9.1/113.4.
32,33. *Syncolporites jardinei* n. fsp., slide: Farafra-6-2-2-4; cross-table number: 3.6/116.1.
34,35. *Syncolporites minor* n. fsp., slide: Farafra-6-2-2-10; cross-table number: 14.7/104.6.
36,37. *Syncolporites minor* n. fsp., slide: Farafra-6-2-2-12; cross-table number: 11.3/105.8.
38,39. *Triporopollenites nointelensis* KEDVES 1970, *Corylaceae*, slide: 70-1-7-1-1; cross-table number: 15.4/109.9.

Stratum typicum: clayey brown coal.

Derivatio nominis: In honour of Dr. A. E. GONZÁLEZ GUZMÁN.

Differential diagnosis: The characteristic verrucate sculpture separates this taxon from *Annutriporites* GONZÁLES GUZMÁN 1967, the exoapertures (short colpi) from *Cranwellipollis* MARTIN et HARRIS 1974.

1. *Guzmanipollenites cretaceus* n. fsp.
(Plate 2.2., figs. 14-19)

Diagnosis: Amb triangular, with straight or slightly convex sides. Surface verrucate. The basal diameter of the sculptural elements is 0.8-1.5 µm. The exine is very thin, about 0.2 µm and the fine structure is not discernible by optical microscopy. Furrows 4-5 µm long and the annulus is 1-1.5 µm wide.

Diameter: 33 µm; 22-38 µm.

Holotype, locus typicus, stratum typicum see at the fgen. type.

Derivatio nominis: From the Cretaceous age.

Differential diagnosis: There are several similarities with *Echitriporites triangulifor-mis* VAN HOEKEN-KLINKENBERG 1964 (Upper Cretaceous, Nigeria and GONZÁLEZ GUZMÁN, 1967, Paleocene, Columbia). A re-examination of the original material of Nigeria is desirable.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-2) infrequent, Farafra (6-2-1) common, Farafra (11) infrequent.

Form-genus: *Scabratriporites* VAN DER HAMMEN 1956

1. *Scabratriporites druggii* n. fsp.
(Plate 2.2., figs. 20-25, 24,25 diporate form)

Diagnosis: Amb triangular, with mildly convex sides. Surface finely scabrate. The inter-apertural exine is 0.2 µm thick. The exine stratification and the structure are not discernible by optical microscopy. Furrows 6-10 µm long, and 1-2 µm wide. The annulus is 1-1.5 µm wide.

Diameter: 23 µm; 20-25 µm.

Holotype: Plate 2.2., figs. 20,21, slide: Farafra-6-2-2-1; cross-table number: 20.2/109.3.

Locus typicus: Farafra, Maestrichtian, Nubia Sandstone.

Stratum typicum: clayey brown coal.

Derivatio nominis: In memoriam of Dr. W. S. DRUGG excellent investigator of the Cretaceous and Lower Tertiary sporomorphs.

Differential diagnosis: *Triporopollenites marcaensis* DRUGG 1967 (Maestrichtian; M. - Danian transition, California, U.S.A.) has a sometimes faintly scrobiculate surface. The surface of the germinal region of *Scabratriporites samoilovichii* BOLTENHAGEN 1976 is finely echinate.

Occurrence and frequency in the samples investigated from Egypt: Lower Campanian: Duwi infrequent; Maestrichtian, Nubia Sandstone: Farafra (6-2-2) common, Farafra (6-2-1) infrequent, Farafra (11) common, Duwi Range (100) infrequent.

2. *Scabratriporites simpliformis* VAN HOEKEN-KLINKENBERG 1966
(Plate 2.2., figs. 26-29)

Diagnosis: Amb triangular, with slightly convex or straight sides. Surface scabrate. The inter-apertural exine is 0.6-0.8 µm thick. The tectum, infratectal layer, and the foot layer are of equal thickness, T/I/F = 1/1/1. The fine structure of the infratectal layer is not discernible by optical microscopy. The furrows are of 6-8 µm long, and are narrow. The annulus is 0.8-1.1 µm in width.

Diameter: 11.5 µm; 10-18 µm.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-2) infrequent, Farafra (6-2-1) infrequent.

Form-genus: *Syncolporites* VAN DER HAMMEN 1954, 1956

1. *Syncolporites jardinei* n. fsp.
(Plate 2.2., figs. 30-33)

Diagnosis: Amb triangular, with straight or mildly convex sides. Surface smooth or scabrate. The inter-apertural exine is 0.2-0.3 µm thick. Its stratification and structure are not discernible by optical microscopy. The furrows are 3-4 µm long, and the annulus is

1-1.3 µm in thickness. There are very characteristic plicae located between the apertural area.

Diameter: 20 µm; 16-24 µm.

Holotype: Plate 2.2., figs. 30,31, slide: Farafra-6-2-2-8; cross-table number: 9.1/113.4.

Locus typicus: Farafra, Maestrichtian, Nubia Sandstone.

Stratum typicum: clayey brown coal.

Derivatio nominis: In honour of Dr. S. JARDINÉ.

Differential diagnosis: The characteristic plicae separates this taxon from *S. incomptus* VAN HOEKEN-KLINKENBERG 1964.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-2) common, Farafra (6-2-1) infrequent, Farafra (11) infrequent.

2. *Syncolporites minor* n. fsp.
(Plate 2.2., figs. 34-37)

Diagnosis: Amb triangular, with slightly convex sides. Surface finely scabrate. The inter-apertural exine is 0.3 µm thick. Its stratification and structure are not discernible by optical microscopy. The furrows are 4-6 µm long, and the annulus is 0.5-0.8 µm in thickness. Usually there are characteristic plicae between the apertures.

Diameter: 12 µm; 9-13 µm.

Holotype. Plate 2.2., figs. 34,35, slide: Farafra-6-2-2-10; cross-table number: 14.7/104.6.

Locus typicus: Farafra, Maestrichtian, Nubia Sandstone.

Stratum typicum: clayey brown coal.

Derivatio nominis: From its smaller size.

Differential diagnosis: The smaller size and the outline of the apertural area distinguishes this taxon from *S. minutus* VAN HOEKEN-KLINKENBERG 1964.

Occurrence and frequency in the samples investigated from Egypt: Maestrichtian, Nubia Sandstone: Farafra (6-2-2) common.

Form-genus: *Triporopollenites* PFLUG et THOMSON 1953

Triporate pollen grains, surface smooth or faintly sculptured.

1. *Triporopollenites nointelensis* KEDVES 1970, *Corylaceae*
(Plate 2.2., figs. 38,39)

Description: Amb triangular, with concave sides. Surface granular to finely rugulate. The inter-apertural exine is 1.5-1.8 µm thick. The tectum, infratectum, and the foot layer are equal. The structure is not clearly discernible by optical microscopy but is probably granular. The annulus is 1.5-2 µm thick and the diameter of the pore is 1-1.5 µm.

Diameter: 27 µm.

Occurrence and frequency in the samples investigated from Egypt: Coniacian-Santonian: Abu Rauwash (70-1-7-1) common.

To be continued

Acknowledgements

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References

BATTEN, D. J. and CHRISTOPHER, R. A. (1981): Key to the recognition of *Normapolles* and some morphologically similar pollen genera. - Rev. Palaeobot. Palynol. *35*, 369-383.

BOLTENHAGEN, E. (1976): Pollen et spores Sénoniens du Gabon. - Cahiers de Micropaléontologie *3*, 1-21.

COOKSON, I.C. (1950): Fossil pollen grains of proteaceous type from Tertiary deposits in Australia. - Australian J. of Sci, Res. B, *3*, 166-177.

COOKSON, I.C. and PIKE, K.M. (1954): Some dicotyledonous pollen types from Cainozoic deposits in the Australian region. - Australian J. of Bot. 2, 197-219.

COUPER, R.A. (1953): Part I.

DETTMANN, M.E. and JARZEN, D.M. (1988): *Angiosperm* pollen from uppermost Cretaceous strata of southeastern Australia and the Antarctica Peninsula. - Mem. Ass. Australasian Paleontologists *5*, 217-237.

DETTMANN, M.E. and JARZEN, D.M. (1996): Pollen of proteaceous-type from latest Cretaceous sediments, southeastern Australia. - Alcheringa *20*, 103-160.

DETTMANN, M.E. and THOMSON, M.R.A. (1987): Cretaceous palynomorphs from the James Ross Island Area, Antarctica - A pilot study. - Br. Antarct. Surv. Bull. *77*, 13-59.

DRUGG, W.S. (1967): Palynology of the Upper Moreno Formation (Late Cretaceous - Paleocene) Escarpado Canyon, California). - Palaeontographica *B, 120*, 1-21.

FREDERIKSEN, N.O. and CHRISTOPHER, R.A. (1978): Taxonomy and biostratigraphy of Late Cretaceous and Paleogene triatriate pollen from South Carolina. - Palynology 2, 113-145.

GLADKOVA, A.N. (1965): Fossil *Myricaceae* of Western Siberia. - Trud. V.N.I.G.R.I. *239*, 142-190 (Russian).

GÓCZÁN, F., GROOT, J.J., KRUTZSCH, W. und PACLTOVÁ, B. (1967): Die Gattungen des "Stemma *Normapolles* PFLUG 1953b" (*Angiospermae*) Neubeschreibungen und Revision europäischer Formen (Oberkreide bis Eozän). - Paläont. Abh. *B*, 2, 427-637.

GÓCZÁN, F., KRUTZSCH, W. und PACLTOVÁ, B. (1967): In: GÓCZÁN, F., GROOT, J.J., KRUTZSCH, W. und PACLTOVÁ, B.

GONZÁLEZ GUZMÁN, A.E. (1967): Part II.

HEGEDÚS, M., KEDVES, M. and PÁRDUTZ, Á. (1971): Ultrastructural investigations on fossil *angiosperm* exines of Upper Cretaceous. - Advancing Frontiers of Plant Sciences *28*, 317-329.

KEDVES, M. (1969): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne. IV. Pollens des *Normapolles*. - Pollen et Spores *11*, 385-396.

KEDVES, M. (1970): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne. V. Pollens triporés, subtriporés et intratriporés. - Pollen et Spores *12*, 83-97.

KEDVES, M. (1974): Part I.

KEDVES, M. (1979): Palynological investigations on sediments of the Lower Danian (Fish Clay, Denmark) I. - Acta Miner.-Petr. Szeged. *24*, 167-186.

KEDVES, M. (1982): In KEDVES and RUSSELL.

KEDVES, M. (1990): Transmission electron microscopy of the fossil *angiosperm* exines. - Szeged.

KEDVES, M. and RADVÁNSZKY, M. (1975): The application of scanning electron microscopical method in some plant microfossils. - Acta Bot. Acad. Sci. Hung. *21*, 51-59.

KEDVES, M. and RUSSELL, D.E. (1982): Palynology of the Thanetian layers of Menat. - Palaeontographica *B, 182*, 105-150.

KRUTZSCH, W. (1959): Einige neue Formgattungen und -Arten von Sporen aus der mitteleuropäischer Oberkreide und dem Tertiär. - Palaeontographica *B, 105*, 125-157.

MARTIN, A.R.H. (1973): Reappraisal of some palynomorphs of supposed proteaceous affinity. I. The genus *Beauprealdites* COOKSON ex COUPER and the species *Proteacidites hakeoides* COUPER. - Geological Society of Australia Special Publication *4*, 73-78.

MARTIN, A.R.H. and HARRIS, W.K. (1974): Reappraisal of some palynomorphs of supposed proteaceous affinity. - Grana *14*, 108-113.

MÉDUS, J. (1975): Fine structure of *Oculopollis parvoculus* GÓCZÁN and *Oculopollis semimaximus* W. KR. - Grana *15*, 113-116.

MÉDUS, J. (1977): Indications de l'ultrastructure d'une espèce de forme du Crétacé supérieur, *Oculopollis maximus* W. KRUTZSCH. - Rev. Palaeobot. Palynol. *24*, 209-215.

MILDENHALL, D.C. (1978): *Cranwellia costata* n.sp. and *Podosporites erugatus* n.sp. from Middle Pliocene (? Early Pleistocene) Sediments, South Island, New Zealand. - Journal of the Royal Society of N.Z. *8*, 253-274.

NAUMOVA, S. N. (1937): Part III.

PETROSJANTZ, M.A. and TROFIMOV, D.M. (1971): Caractéristiques sporo-polliniques des formations du Crétacé supérieur de la synéclise du Niger, Mali (Sahara méridional). - Biul. Moskov. Obs. Isp. Prirod. Otd. Geol. *76*, 75-85 (Russian).

PETROSJANTZ, M.A. and TROFIMOV, D.M. (1975): Palynologic characteristics of the Danian-Paleocene layers of South-West Sahara (Synclin Tauden and Mali-Niger). - Izv. Acad. Nauk. USSR *5*, 108-115 (Russian).

PFLUG, H.D. (1953a): In THOMSON und PFLUG, 1953.

PFLUG, H.D. (1953b): Part I.

PFLUG, H.D. und THOMSON, P.W. (1953): Part IV.

POTONIÉ, R. (1960): Part III.

POTONIÉ, R. (1970): Synopsis der Gattungen der Sporae dispersae V. Teil: Nachträge zu allen Gruppen (Turmae). - Beih. geol. Jb. *87*, 1-222.

THOMSON, P.W. und PFLUG, H.D. (1953): Pollen und Sporen des mitteleuropäischen Tertiärs. - Palaeontographica *B, 94*, 1-128.

VAN DER HAMMEN, TH. (1954): El desarollo de la Flora Colombiana en los periodos geologicos I: Maestrichtiana hasta Terciario mas Inferior. - Boletin Geologico 2, 49-106.

VAN DER HAMMEN, TH. (1956): Part III.

VAN HOEKEN-KLINKENBERG, P.M.J. (1964) Part I:

VAN HOEKEN-KLINKENBERG, P.M.J. (1966): Part III.

VARMA, C. P. and RAWAT, M. S. (1963): A note on some diporate grains recovered from Tertiary horizons of India and their potential marker value. – Grana Palynologica *4*, 130-139.

3. WOOD ANATOMY OF A SILICIFIED TRUNK FROM THE KHARGA OASIS, EGYPT

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Abstract

This paper presents the detailed LM anatomy of a gymnospermous fossil wood from the Late Cretaceous layers of the Kharga Oasis. The pitting of the radial wall of the tracheids is of an early "araucarioid" type. In comparison to the previous fossil wood data the following anatomical characteristics may be emphasized: 1. The ray cells are 3 - 52 cells high. 2. The great number (21, 24) of the pits in the cross fields. 3. The transverse walls of the longitudinal parenchyma cells are smooth. Based on these LM characteristic features this fossil wood is described as *Agathoxylon lifiyii* sp. nov.

Key words: Xylotomy, fossil, *Agathoxylon*, Upper Cretaceous, Egypt.

Introduction

Four genera and seven species of petrified *gymnosperm* wood have, so far, been reported from Egypt (in addition to unidentified specimens) as tabulated below:

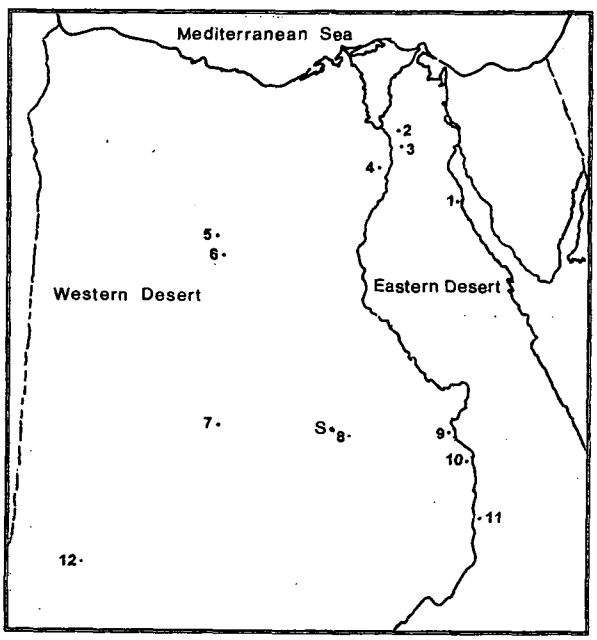
Taxa	Permian Jurassic	Late Cretaceous	Tertiary (Oligocene)	Site	Reference
Family: Araucariaceae					
Dadoxylon: Endlicher 1847					
D. aegyptiacum Unger 1858		+		1,5,6,7,8,9,10	Unger, 1858-59
D. dallonii Boureau 1948	+			12	Duperon-L&Lejal-N., 1981
D. mokattamensis Kräusel 1924			+	3	Kräusel, 1924
D. sudanense Duperon-L.&Lejal-N., 1981	+			12	Duperon-L&Lejal-N., 1981
Family: Cupressaceae					
Cupressinoxylon Goeppert 1850					
?C. sp Kräusel 1924		+		5,6	Kräusel, 1924-39
Family: Podocarpaceae					
Mesembrioxylon (Podocarpoxylon)					
aegyptiacum (Kräusel) Boureau 1950			+	3	Kräusel, 1939
Protophyllocladoxylon Kräusel 1939					
P. leuchsi Kräusel 1939		+		7,8	Kräusel, 1939
Unidentified gymnosperm wood		+		11	Youssef, 1993

For names and locations of these sites see map in text-fig. 3.1.

The aim of this paper is to report on the occurrence of a Late Cretaceous petrified trunk of a *gymnosperm* tree, the xylotomy of which is quite distinct from, not only all those described earlier from Egypt but also from all those described in the available literature from other parts of the world.

The study area

The study area (Text-fig. 3.1.) lies about 12 km to the north-west of Kharga Oasis and about 12 km to the north of bench mark 15 on the Kharga-Dakla Oases road, and stretches almost till Gebel Abo-Tartour (Wadi El-Lifiya). This Late Cretaceous area is covered by Nubia Sandstone (G.S.E., 1982). The fossil woods lie horizontally on the surface and are in the form of large tree trunks (12 trunks were counted) and many fragments of variable size. Most trunks are about 15 m long and 20-35 cm in diameter. No branches, roots, fruits, leaves were found. The woods are highly silificied and variously coloured from white to dark.



Text-fig. 3.1.

Map showing sites of collection of gymnospermous fossil woods in Egypt.

1 - Wadi Araba. 2 - Gebel Ahmar. 3 - Gebel Mokattam. 4 - Gebel Qatrani. 5 - Bahariya Oasis. 6 - Gebel Hufhuf. 7 - Dakhla Oasis. 8 - Kharga Oasis. 9 - Esna. 10 - Road between Esna and Wadi Haifa. 11 - Aswan Area. 12 - Gilf Kebir.

S - Study Area.

Materials and Methods

Twelve specimens were chopped off from the 12 large trunks. The majority of specimens range between 5-10 cm in width and 10-20 cm in length. Thin-ground sections (T.S., T.L.S. and R.L.S.) were prepared (e.g.: cf. LACEY, 1963) from all collected specimens. They all proved to belong to *gymnosperms*. However, only four specimens were found to be well preserved and permit identification to generic or lower level. Only one of these will be described here, while the other three will be the subject of forthcoming publications. The following basic publications were used: GREGUSS (1955, 1958, 1967, 1968a,b, 1972), JANE (1970), MÄGDEFRAU (1953), MOLISCH (1954), SAID (1990).

The specimens and slides are deposited in the paleobotanical collection of the Department of Botany, University of Zagazig, Benha, Egypt.

Results

The specimen to be described here was taken from a tree trunk about 15 m long and 20 cm in diameter.

Cross section (Plate 3.1., figs. 1,2)

The secondary wood is made of tracheids, ray parenchyma, vertical resin ducts and xylem parenchyma, and sometimes traumatic tissue (Plate 3.1., figs. 1,2). Vertical resin ducts are usually present in tangential bands in late wood (Plate 3.1., figs. 1,2) and sometimes found solitary within the early wood: 74-133 µm in diameter. Walls of the resin ducts are thick; 15 - 44 µm in thickness. Annual rings distinct, variable in width from 375 to 1875 µm (Plate 3.1., fig. 1; text-fig. 3.2.). Transition from early to late wood is gradual in the wider rings and abrupt in the narrower rings; the late wood is very narrow from 2 to about 4 cells. Discontinuous rings sometimes present (Plate 3.1., fig. 1). Tracheids in early wood quadrate, spherical and elliptical; 32 - 72 µm and 36 - 65 µm in radial and tangential diameters respectively; thick walled; 4 - 7 µm. Late woods tracheids smaller in radial diameter: 18-36 µm with thick walls; 4-11 µm.

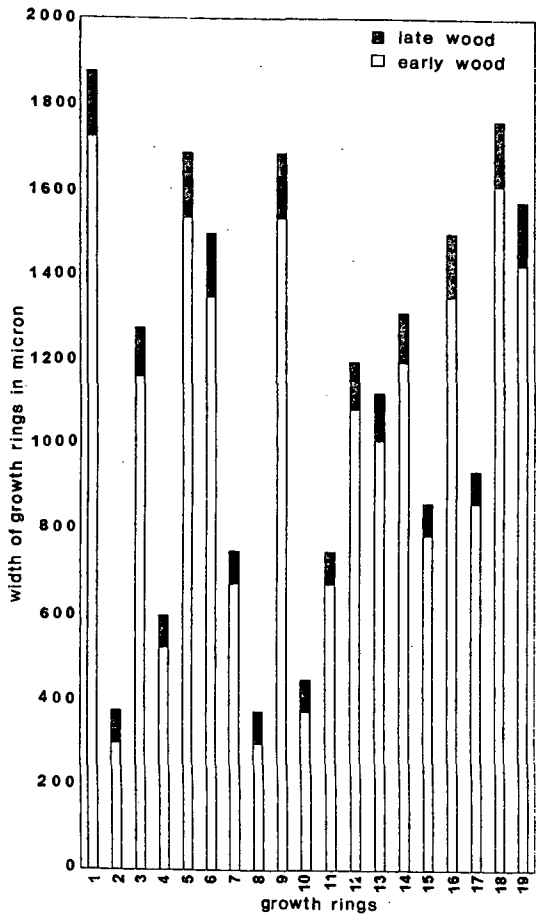
Tangential section (Plate 3.2., figs. 1- 5)

The rays are uniseriate and 3 - 52 cells high. The preservation of the wood was not so well for quantitative evaluation of the number of the ray cells. The cell number of 99 rays was counted, the greatest part, 75 rays were between 3 - 19 cells high, and 24 were 20 - 52 cells high. The cross section of the cells is sometimes deformed by fossilization, they are mostly squares, circles or upright ellipses, 14-22 µm in diameter. The horizontal wall of the longitudinal parenchyma is smooth. There are dark resinous remnants in these cells. (Plate 3.2., figs. 3-5).

Radial section (Plate 3.2., figs. 6-9, plate 3.3., figs. 1-5)

The areolate pits of the radial walls of the tracheids uni- or biseriate (Plate 3.3., figs. 1-4) with round apertures, 7-11 µm. Typically araucarioid pitting is well shown in figs. 1 and 2 on Plate 3.3. In the cross fields there are several (21, 24) simple pits, arranged in two or four but mostly in three rows. Generally in the late wood, spiral wall structure of the tracheids was observed (Plate 3.3., fig. 5). This kind of thickening was discussed by GREGUSS (1967) and HUARD (1966), but this seems to be an early characteristic (cf. SIMONCSICS, 1955).

Taking into consideration the previously described wood anatomical characteristic features, this fossil wood is a new "araucarioid type" for the literature.



Text-fig. 3.2.

Showing variation in width of growth rings (early and late wood).

Agathoxylon lifiyii sp. nov. (Plate 3.1 - 3.3)

Diagnosis

Secondary wood is homoxyl, with vertical resin ducts, and annual rings. The rays are 3 - 52 cells high, the greatest part until 19 cells. The areolate pits of the radial wall are typically early, araucarioid type. The number of the simple pits in the cross fields is high; 21, 24, arranged into 2 - 3 - 4 rows. Spiral wall structure of the tracheids occurred in general in the late wood.

Holotype: Deposited in the Department of Botany of the Zagazig University, Benha, Egypt.

Locus typicus: Kharga-Dakhla Oases.

Stratum typicum: Late Cretaceous, Nubia Sandstone.

Derivatio nominis: From Wadi Lifiya, the site of the collection.

Differential diagnosis: The relatively high number of the ray cells and the simple pits in the cross fields separate this wood well from the similar fossil woods of *Araucariaceae,* namely from *Agathoxylon hungaricum* (ANDREÁNSZKY 1949) GREGUSS 1952,

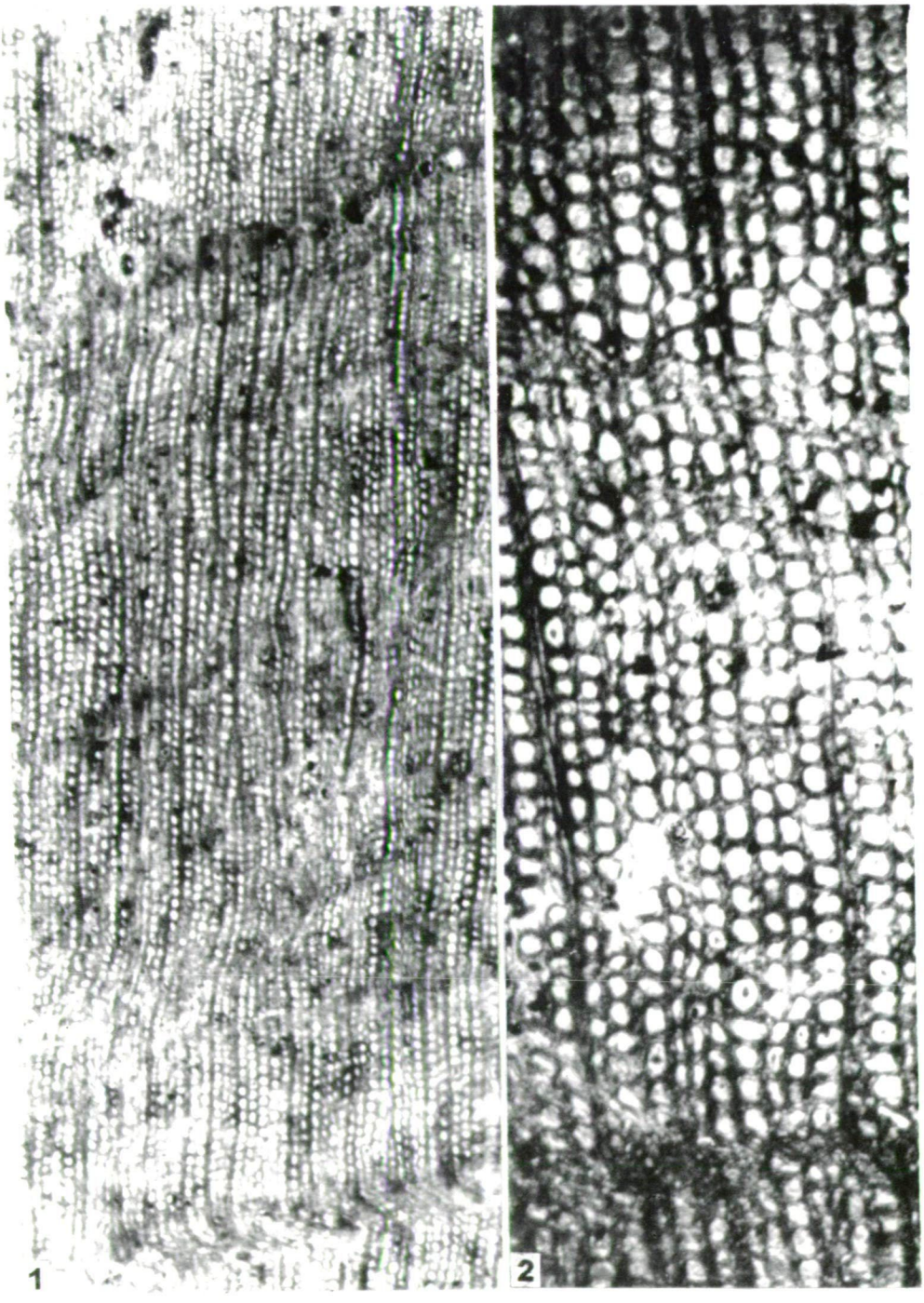


Plate 3.1.

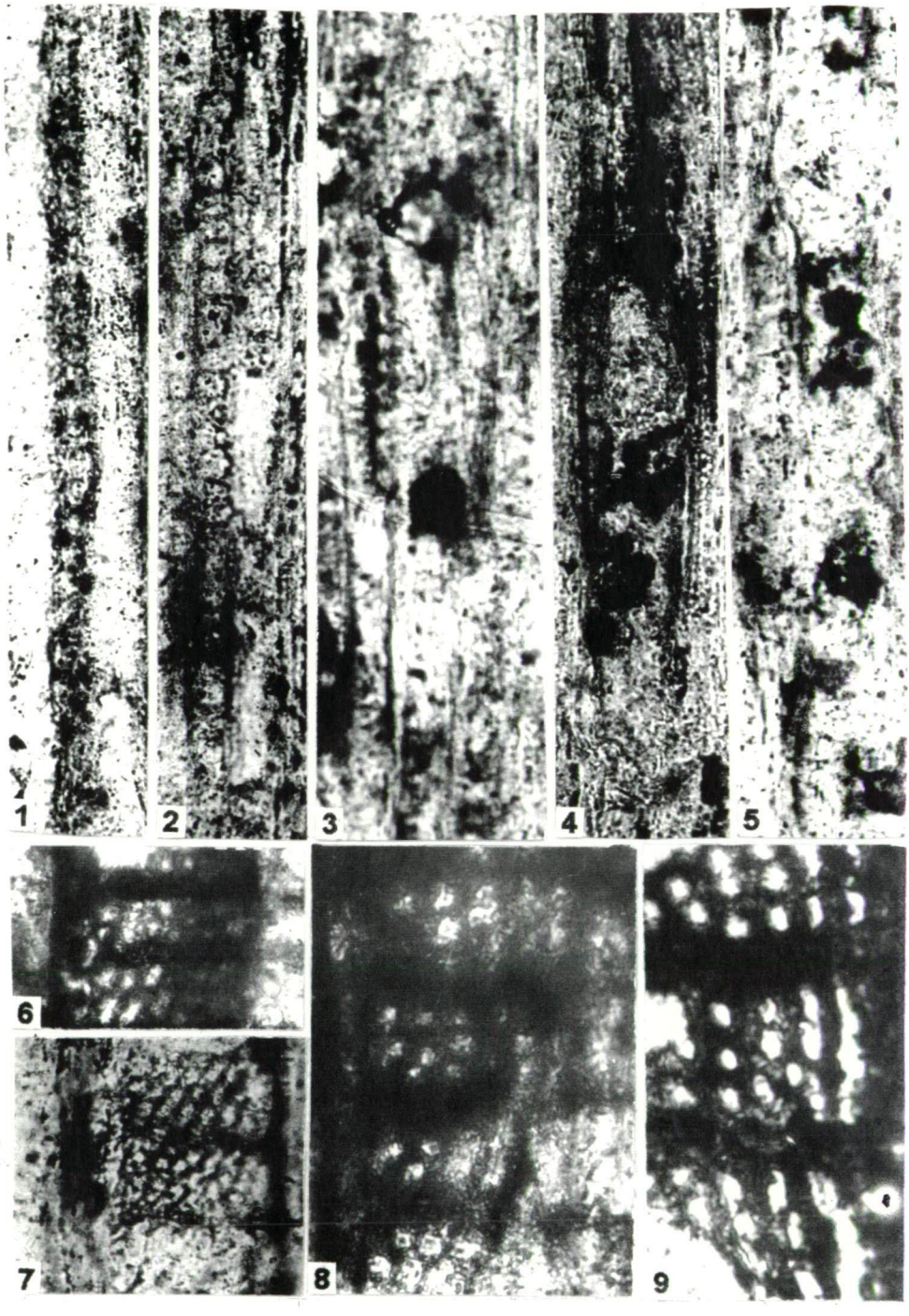


Plate 3.2.

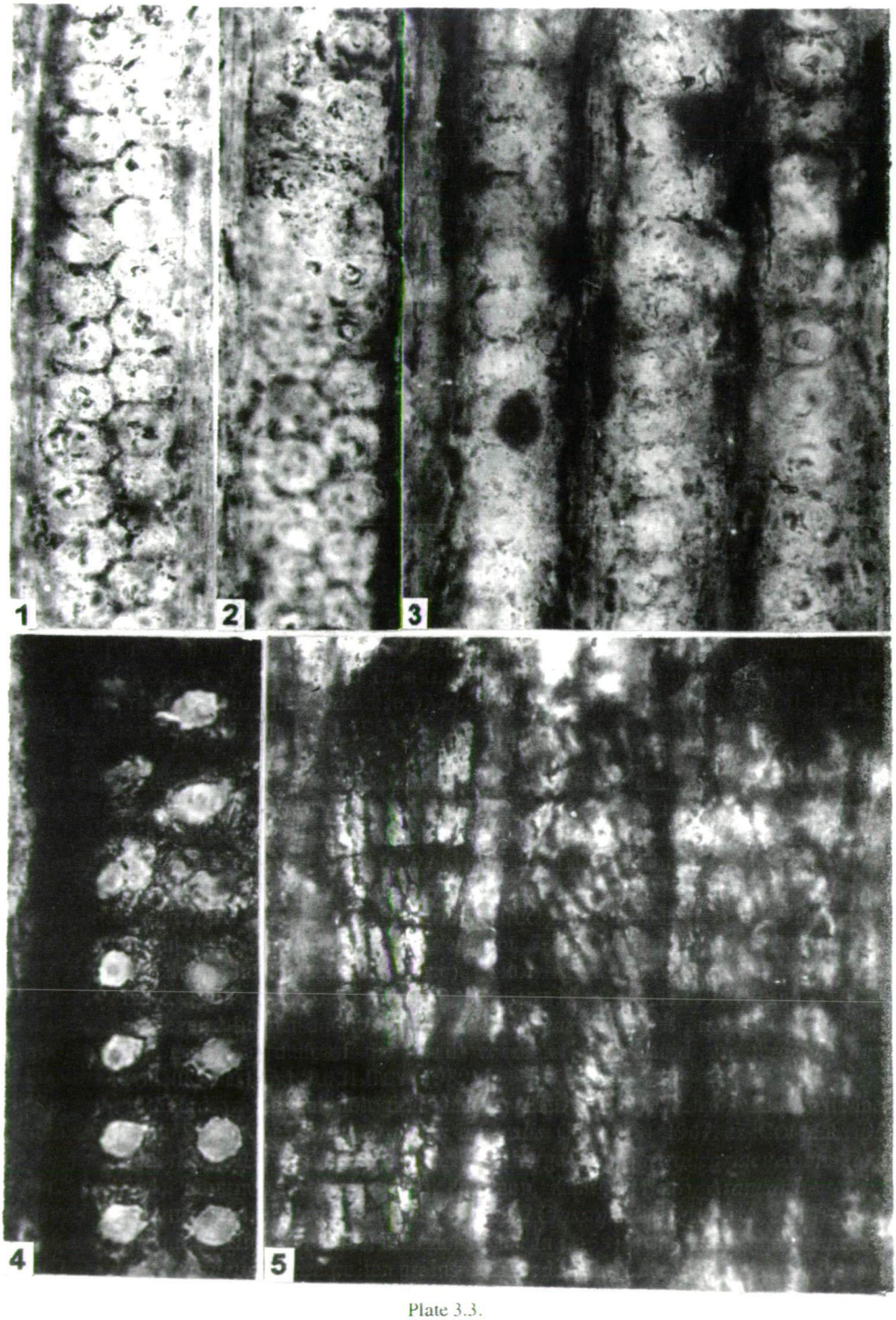


Plate 3.3.

Plate 3.1.

1,2. *Agathoxylon lifiyii* n. fsp., cross section.
1. Showing growth rings, position of resin ducts and diffuse xylem parenchyma with dark contents. 25x.
2. Annual rings, rays with dark contents and xylem parenchyma. 100x.

Plate 3.2.

1-9. *Agathoxylon lifiyii* n. fsp.
1-5. Tangential sections, uniseriate long rays. 250x.
3,4. Xylem parenchyma with dark drops. 250x.
6-9. Radial section, pitting of the cross fields.
6,7. 500x.
8,9. 1000x.

Plate 3.3.

1-5. *Agathoxylon lifiyii* n. fsp., radial sections.
1,2. Typical biseriate areolate araucarioid pits of the radial wall of the tracheids. 500x.
3. Uniseriate areolate pits of the radial wall of the tracheids. 500x.
4. Biseriate pits of the radial wall of the tracheids. 500x.
5. Cross fields of the late wood, showing peculiar spiral thickenings of the tracheids. 500x.

Araucarioxylon resiniferum GREGUSS 1967 and *Dadoxylon agathiforme* KEDVES 1955. The number of the cross-field pits of the early types is less than the described n. sp., but the spiral wall structure of the tracheids is present; cf. *Dadoxylon schrollianum* GOEPPERT and *D. transdanubicum* SIMONCSICS 1955, from Permian sediments; cf. GREGUSS (1961, 1967). This kind of spiral structure of the tracheids is known from Cretaceous fossil woods also: *Dadoxylon implexum* ZIMMERMANN 1953 and *D. graminovillae* ZIMMERMANN 1953.

Remarks. - Araucarioid pitting was published previously from different ages and taxa. MÄGDEFRAU (1953) published the Permian (Rotliegendes) *Dadoxylon* remnant as *Cordaites* wood. GOULD (1971) and TAYLOR (1981) published as *Cycadaceae* fossil the *Lyssoxylon grigsbyi* GOULD 1971 from the Triassic Chinle Formation, Arizona. ALVIN et al. (1981) and ALVIN (1982) based on *Pseudofrenelopsis parceramosa* the *Cheirolepidaceous* wood is of araucarioid structure. BARALE et al. (1991) published araucarioid secondary wood structure from *Brachyphyllum trautii* BARALE and CONTINI 1973 from the Bajocian (Jurassic). Following ANDREÁNSZKY (1949, 1954), GREGUSS (1952, 1967), KEDVES (1955), BERTOLANI MARCHETTI (1963), and STOCKEY (1982) and several further authors, the *Araucariaceae* affinity is probable. The most important bibliographical data of the fossil woods of araucarioid structure was reviewed by RAJANIKANTH and SUKH-DEV (1989).

Regarding the palynological data by KEDVES (1997) from Kharga (Maestrichtian, Nubia Sandstone) *Araucariacites australis* COOKSON 1947 ex COUPER 1953 subfsp. *aegypticus* KEDVES 1997, infrequent, Kharga (1-28), *Araucariacites hungaricus* DEÁK 1964, common, Kharga (1-39), common, Kharga (1-28), *Araucariacites balinkaense* KEDVES 1974, infrequent, Kharga (1-39), *Classopollis perplexus* BOLTENHAGEN 1973, infrequent, Kharga (1-39), common, Kharga (1-39). In this way the *Araucariaceae* and the *Cheirolepidaceae* pollen grains occurred in the Late Cretaceous of the Nubia Sand-

stone of Kharga. The botanical affinity of the fossil wood remnant presented herein may be the *Araucariaceae* or *Cheirolepidaceae.*

Beside all that is mentioned above the climate of Egypt in Cretaceous time was warm with dry and rainy seasons (SAID, 1990), which may be comparable to the climate of the area of the extant species of the family *Araucariaceae.*

Discussion and Conclusions

In conclusion it may be said that there are now 3 species of gymnospermous wood reported from the road between Kharga-Dakhla Oases namely: *Dadoxylon aegyptiacum, Protophyllocladoxylon leuchsi,* and *Agathoxylon lifiyii,* in addition to nine species of angiospermous wood namely: *Celastrinoxylon celastroides (Celastraceae), Detarioxylon aegyptiacum (Leguminosae), Ebenoxylon ebenoides (Ebenaceae), Ficoxylon sp. (Moraceae), Hibiscoxylon niloticum (Malvaceae), Proteoxylon khargaense (Proteaceae), Terminalioxylon intermedium (Combretaceae) Ternstroemioxylon dakhlaense (Ternstroemiaceae)* and *Palmoxylon zitteli (Palmae).*

KOENIGUER (1971) published Dicotyledonous woody remnants from the Paleocene layers of Sessao (Niger). It is worth of mentioning, that from the Upper Senonian of Sanhirer BUSSON (1967) published a *Dadoxylon* sp. (type araucarioid) and KOENIGUER (1967) the *Euphorbioxylon bussonii* n sp.

References

ALVIN, K.L. (1982): *Cheirolepidaceae:* Biology, structure and paleoecology, - Rev. Palaeobot. Palynol. *37*, 71-98.

ALVIN, K.L., FRASER, C.J. and SPICER, R.A. (1981): Anatomy and palaeoecology of *Pseudofrenelopsis* and associated conifers in the English Wealden. – Palaeontology *21*, 847-856.

ANDREÁNSZKY, G. (1949): Alsókrétakorú fatörzsek. - Földt. Közl. *79*, 243-252.

ANDREÁNSZKY, G. (1954): Ősnövénytan. - Akadémiai Kiadó, Budapest.

BARALE, G. et CONTINI, D. (1973): La paléoflore continentale du Bajocien franc-comtois. Étude stratigraphique et paléobotanique du gisement de pont-les-Moulins (Doubs). - Ann. Sci. Univ. Besançon *3*, 247-255.

BARALE, G., PHILIPPE, M. et THEVENARD, F. (1991): L'Approche morphologique en Paléobotanique: Application à l'étude du Jurassique. - Geobios *13*, 57-67.

BERTOLANI MARCHETTI, D. (1963): Un legno di conifera silicizzado nelle argille scagliose del preappennino emiliano. - Ann. di Botanica *26*, 405-411.

BOLTENHAGEN, E. (1973): Quelques espèces du genre *Classopolis* (PFLUG) REYRE du Crétacé supérieur du Gabon. – Rev. de Micropaléontologie *16*, 205-213.

BOREAU, E. (1950): Etude paléoxylologique du Sahara (VIII). Sur un échantillon de *Mesembrioxylon aegyptiacum* (KRÄUSEL) BOUREAU n. comb., *Podocarpacée* découverte au Sud de Toummoo (Sahara oriental). – Bull. Mus. Natl. Hist. Nat., 2e sér., *22*, 411-419.

BOUREAU, E. (1958): Paléobotanique africaine. Evolution des flores disparues de l'Afrique nord-équatoriale. – Paris, Bull. Sci. Du comité des travaux historiques et scientifiques, Gauthier-Villars éditeur, *3*:1, 1-63, 8 planches hors-texte.

BUSSON, G. (1967): Le Mésozoique saharien, I: L'Extréme Sud Tunisien. - Publ. Centre Rech. Zones Arides, Ser. Geol. *8*, 1-194.

COOKSON, I.C. (1947): Plant microfossils from the lignites of Kerguelen Archipelago. - B.A.N.Z. Antarctic Research Expedition 1929-1931, Repts A *II/8*, 127-142.

COUPER, R.A. (1953): Upper Mesozoic and Cainozoic spores and pollen grains from New Zealand. - N.Z. Geol. Surv. Paleont. Bull. *22*, 1-77.

DEÁK, H.M. (1964): A Dunántúli Középhegység apti üledékeinek palynológiai vizsgálata (Recherches palynologiques des dépôts aptiens de la Montagne Centrale de Transdanubie). - Geol. Hung. Ser. Palaeontologica *29-32*, 9-105.

DUPÉRON-LAUDOUENEIX, M. et LEJAL-NICOL, A. (1981): Sur deux bois homoxylés du Sud-Ouest de l'Egypte. C. R. 106e Congr. Nat. Soc. Sav., Perpignan, Sci. *1*, 29-40.

Geological Survey of Egypt (1982): Dakhla sheet, scale 1: 250000.

GOULD, R.E. (1971): *Lyssoxylon grigsbyi*, a cycad trunk from the Upper Triassic of Arizona and New Mexico. - Amer. J. Bot. *58*, 239-248.

GREGUSS, P. (1952): Magyarországi *mezozói famaradványok*. - Földt. Közl. *82*, 157-180.

GREGUSS, P. (1955): Xylotomische Bestimmung der heute lebenden Gymnospermen. - Akadémiai Kiadó, Budapest.

GREGUSS, P. (1958): Xylotomische Bestimmung der Koniferen-Familien. - Wissenschaftliche Zeitschrift der pädagogischen Hochschule Potsdam Math.-Naturw. Reihe *3*, 165-180.

GREGUSS, P. (1961): Permische fossile Hölzer aus Ungarn. - Palaeontographica B, *109*, 131-146.

GREGUSS, P. (1967): Fossil *gymnosperm* woods in Hungary from the Permian to the Pliocene. - Akadémiai Kiadó, Budapest.

GREGUSS, P. (1968a): Einführung in die Paläoxylotomie. - Geologie *17*, BH 60, 1-87.

GREGUSS, P. (1968b): Xylotomy of the living Cycads. - Akadémiai Kiadó, Budapest.

GREGUSS, P. (1972): Xylotomy of the living Conifers. - Akadémiai Kiadó, Budapest.

HUARD, J. (1966): Étude anatomique des bois de Conifères des couches à lignite Néogènes des Landes. - Mém. Soc. Géol. de France N.S. *105*, 5-85.

JANE, F.W. (1970): The structure of wood. - A. and C. Black, LTD, London.

KEDVES, M. (1955): Egy kovásodott fatörzsmaradvány xylotomiai vizsgálata. - Tudományos Diákköri Dolgozatok *II./b.*, 3-7.

KEDVES, M. (1974): Paleogene fossil sporomorphs of the Bakony Mountains Part II. - Studia Biol. Acad. Sci. Hung. *13*, 1-14.

KEDVES, M. (1997): Upper Cretaceous pollen grains from Egypt. I. - Plant Cell Biology and Development (Szeged) *8*, 10-33.

KOENIGUER, J.C. (1967): Sur la présence d'une *euphorbiacée* fossile dans le Sénonien supérieur du Sud-Tunisien: *Euphorbioxylon bussonii* n. sp. - The Palaeobotanist. *16*, 170-176.

KOENIGUER, J.C. (1971): Sur les bois fossiles du Paléocène de Sessao (Niger). - Rev. Palaeobotan. Palynol. *12*, 303-323.

KRÄUSEL, R. (1924): Ergebnisse der Forschungsreisen Prof. E. STROMERS in den Wüsten Ägyptens. IV. (1). (2), (3); (A) *Fungi, Algae*, (B) *Gymnospermae, Coniferae*, (C) *Angiospermae, Monocotyledonae*. – Abh. Bayer. Acad. Wiss., München *30*, 1-48.

KRÄUSEL, R. (1939): Ergebnisse der Forschungsreisen Prof. E. STROMERS in den Wüsten Ägyptens. 3. Die Fossilen Pflanzen Ägyptens, E-L. – Abh. Bayer. Akad. Wiss. München (N. F.) *47*, 1-140.

LACEY, W.S. (1963): Palaeobotany Technique, View Points in Biology. J.D. CARTHEY and I. DUDDINGTON eds. *2*, 202-243.

MÄGDEFRAU, K. (1953): Paläobiologie der Pflanzen. - Gustav Fischer Verlag, Jena.

MOLISCH, H. (1954): Anatomie der Pflanzen. - VEB Gustav Fischer, Jena.

PHILIPPE, M. (1993): Nomenclature génériques des trachéidoxyles fossiles mésozoïques à champs araucarioïdes. – Taxon *42*, 74-80.

RAJANIKANTH, A. and SUKH-DEV (1989): The Kota Formation: Fossil flora and stratigraphy. - Geophytology *19*, 52-64.

SAID, R. (1990): The geology of Egypt. Published for the Egyptian General Petroleum Corporation Conoco Hurghada Inc. and repsol Exploration, S.A. - A.A. Balkema, Rotterdam/Brookfield.

SIMONCSICS, P. (1955): Verkieselte permische Stammreste von dem Mecsek Gebirge. - Acta Biol. Szeged. *1*, 46-62.

STOCKEY, R.A. (1982): The Araucariaceae: An evolutionary perspective. - Rev. Palaeobot., Palynol. *37*, 133-154.

TAYLOR, T.N. (1981): Paleobotany An Introduction to Fossil Plant Biology. - McGraw- Hill Inc., U.S.A.

UNGER, F. (1858-1859): Der versteinerte Wald bei Kairo und einige andere verkieselten Holzes in Ägypten. - Sitz- Ber. K. Akad. Wiss. M.N. Kl *33*, 209-233.

YOUSSEF, S.M. (1993): Studies on some Egyptian fossil woods. - PhD Thesis, Fac. Sci. Univ. Zagazig, Benha, Egypt.

ZIMMERMANN, G. (1953): Anatomische Untersuchungen an Kieselhölzer aus dem Stubenstandstein Würtenbergs. – Palaeontographica B, 93.

4. TRANSMISSION ELECTRON MICROSCOPY OF THE PARTIALLY DEGRADED POLLEN GRAINS FROM THE THANETIAN LAYERS OF MENAT (FRANCE) I.

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Abstract

Gymnosperm and *angiosperm* pollen grains were partially degraded with merkaptoethanol during 2.5 and 5 hours, and investigated with the TEM method. The saccate *gymnosperm* pollen grains *(Pityosporites alatus, P. pristinipollinius)* are relatively resistant against the experimental and embedding processes. The tectum and the infratectal layer at the investigated *Longaxones (Monocolpopollenites tranquillus, Cupuliferoidaepollenites quisqualis, C. liblarensis, Cupuliferoipollenites insleyanus, C. oviformis, C. pusillus, Retitricolpites thomsonii)* taxa are also resistant. The granular infratectal layer of the two *Normapolles (Plicapollis, Stephanoporopollenites)* taxa is less resistant, in opposition to this, the granular infratectal layer of the *Platycaryapollenites (Postnormapolles)* is well preserved after the partial dissolution experiment. The resistance of the pollen grains of the recent *Platycarya* against different kinds of experimental influences was emphasized previously. The ultrastructure of the pollen grains embedding organic material was also investigated and interpreted.

Key words: Palynology, Paleocene, Menat, France, experimental ultrastructure.

Introduction

EHRLICH and HALL (1959) in its pionnering paper on the transmission electron microscopy of fossil pollen grains among others called attention to the preservation of the ultrastructure of the fossil organic material. KEDVES, STANLEY and ROJIK (1974) observed the partial degradation of fossil pollen grains during fossilization processes from some *angiosperm* pollen grains isolated from the Eocene layers of Missisippi. Larger globular molecular structures were published from the infratectal layer of *Restioniidites hungaricus* (KEDVES 1965) ELSIK 1968 and from the ectexine of *Thomsonipollis magnificus* (PFLUG and THOMSON 1953) KRUTZSCH 1960. In several papers it was emphasized, that the preservation of the fossil organic material depends from several factors, such the basic chemistry of the organic material, the sedimentation processes, after the treatment of the sediments, the experiments and the fixation and embedding processes for transmission electron microscopical investigations. ROWLEY and SRIVASTAVA (1986) investigated the exine of *Classopollis* after oxidative etching. Biopolymer structure of this pollen grain was also established. Later ROWLEY, J.R., ROWLEY, J.S. and SKVARLA (1990) described the ultrastructure of the corroded exines from the HAVINGA's experiments.

The Paleocene (Thanetian) layers of Menat are very rich in well preserved sporomorphs. Some previous papers, and the monographical elaboration of this spore-pollen assemblage was previously published by KEDVES and RUSSELL (1982). Later some ultrastructural data from the partially degraded pollen grains from Menat were published (KEDVES, 1986, 1988) and two kinds of experiments were made on this material but till this time without systematic study and publication.

The aim of this paper is to investigate in detail the ultrastructure results of the so-called moderatedly degraded pollen grains in comparison to other fossil and recent pollen data.

Materials and Methods

The locality was described previously by RUSSELL (1982) in KEDVES and RUSSELL. The sediments were treated with HCl and HF. The organic residue was dried. 20 mg organic material was used for all experiments. 1 ml merkaptoethanol was added for the dry organic material and the length of time was 2.5 and 5 hours. For TEM studies the washed material was postfixed with OsO_4 aq.dil. and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made with glass knives, the TEM pictures were taken with a Tesla BS 500 instrument, resolution 5 Å.

The investigated pollen taxa with the length of time and the block numbers are as follows:

Pityosporites alatus (2.5h: 85/48), *Pityosporites pristinipollinius* (2.5h: 85/45, 5h: 85/36), *Monocolpopollenites tranquillus* (2.5h: 85/51), *Cupuliferoidaepollenites quisqualis* (2.5h: 85/64, 85/66, 86/4, 5h: 85/8, 85/37). *Cupuliferoidaepollenites liblarensis* (2.5h: 85/49, 85/60b, 86/6, 5h: 85/41, 86/2), *Cupuliferoipollenites insleyanus* (2.5h: 85/63), *Cupuliferoipollenites oviformis* (2.5h: 85/55, 5h: 85/35), *Cupuliferoipollenites pusillus* (2.5h: 85/31, 85/56, 85/57, 85/58, 5h: 85/38), *Retitricolpites thomsonii* (2.5h: 85/62, 86/1, 5h: 85/40, 85/42, 86/3), *Stephanoporopollenites hexaradiatus* (2.5h: 85/61), *Plicapollis pseudoexcelsus* (2.5h: 85/47), *Platycaryapollenites platycaryoides* (2.5h: 85/54, 85/60a, 85/65).

Results

Gymnosperm pollen grains

Two saccate *gymnosperm* pollen grains was investigated:

1. *Pityosporites alatus* (POTONIÉ 1931) THOMSON et PFLUG 1953, *Abietaceae, Pinus* (Plate 4.1.)

2. *Pityosporites pristinipollinius* (TRAVERSE 1955) KRUTZSCH 1971, *Abietaceae, Pinus* (Plate 4.2., figs. 1,2)

Remark. - The ultrastructure of fossil saccate *gymnosperm* pollen grains was summarized in the monograph of KEDVES (1994).

Based on our present results, the molecular system of the sporopollenin of the saccate *gymnosperm* pollen grains is resistant. The alveolar ectexine of the corpus is well shown in Plate 4.2., fig. 2, the saccus is represented on both investigated species (Plate 4.1., and Plate 4.2., fig. 1). The different kinds of alveoli (a_1, a_2, a_3) are well shown. On the other hand it is worth mentioning, that the outer surface is covered with organic material with fine lamellar or spongy ultrastructure. There are globular electron dense particles

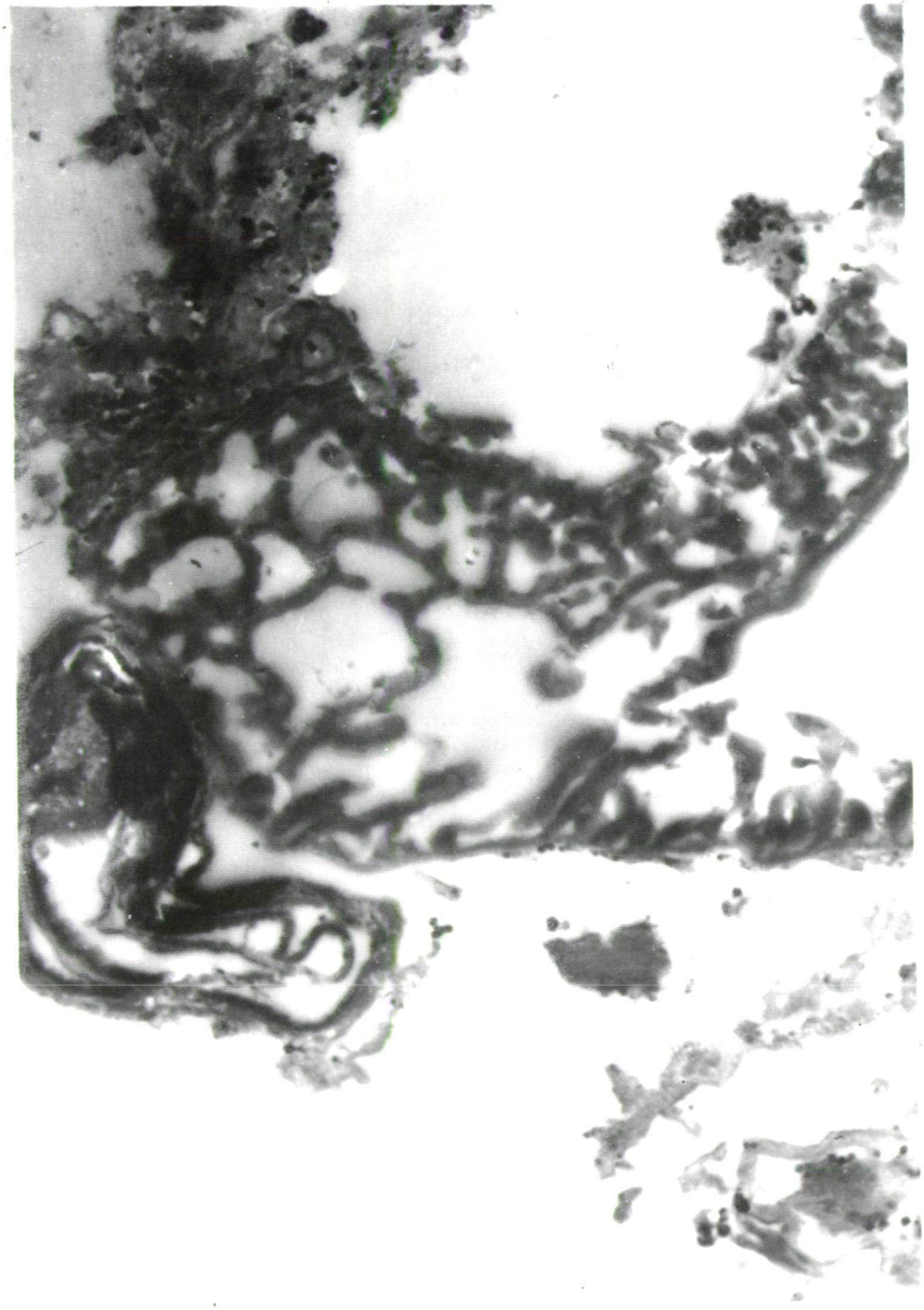

Plate 4.1.

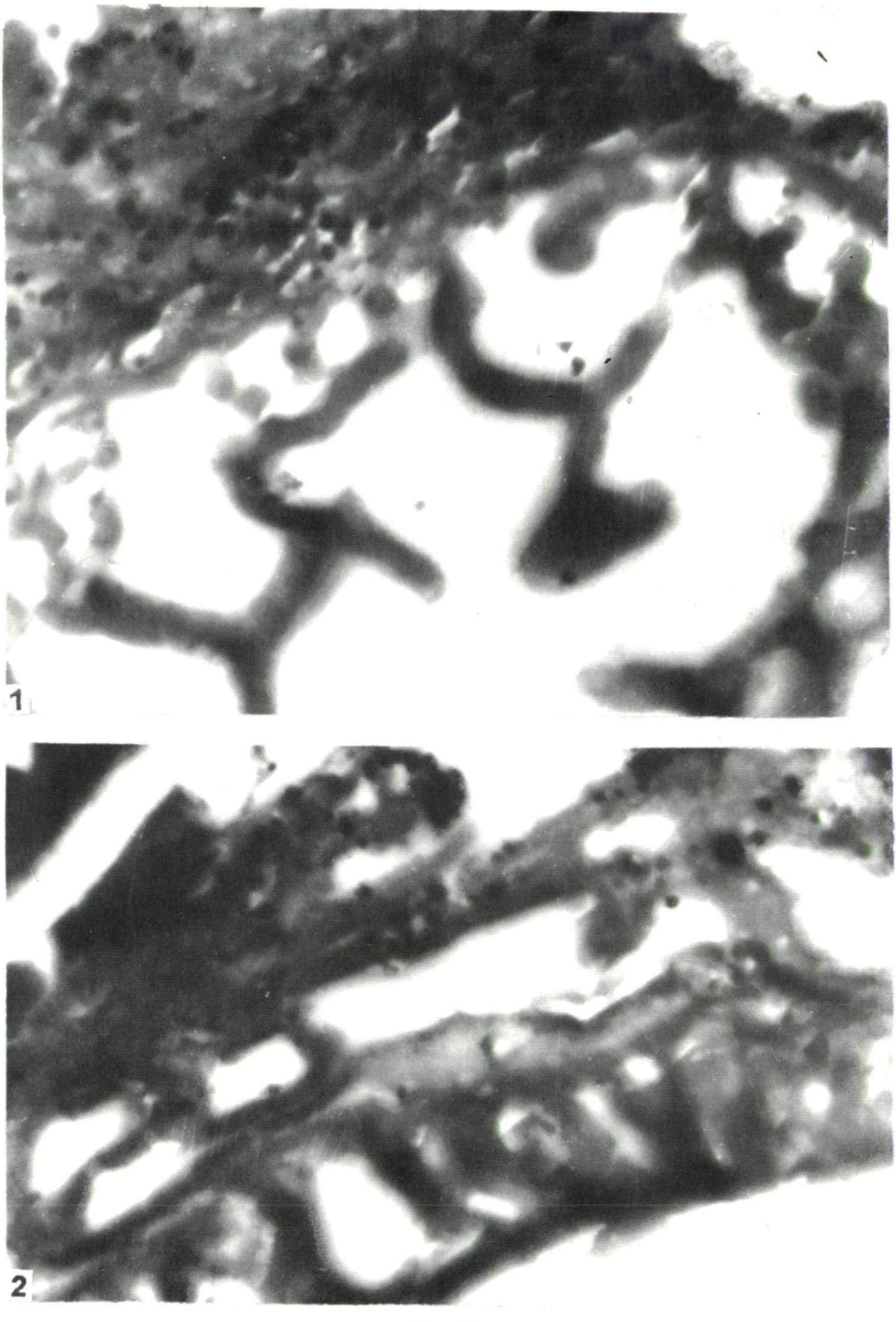

Plate 4.2.

Plate 4.1.

Pityosporites alatus (POTONIÉ 1931) THOMSON et PFLUG 1953, *Abietaceae, Pinus*, block number: 85/45, negative no: 5340, 10.000x.

Plate 4.2.

1,2. *Pityosporites pristinipollinius* (TRAVERSE 1955) KRUTZSCH 1971, *Abietaceae, Pinus*, block number: 85/48.
1. Negative no: 5346, 25.000x.
2. Negative no: 5344, 25.000x.

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within the spongy and/or lamellar organic matrix. Similar ultrastructure was published by GLIKSON and TAYLOR (1986), p. 276, fig. B,C, p. 272: "bacterial remains as a part of main component of vitrinite-like organic matter (TEM)," But this embedding matrix may be also in consequence of the electrostatic charge of the surface.

Angiosperm pollen grains
Longaxones

Monocolpopollenites tranquillus (POTONIÉ 1934) THOMSON et PFLUG 1953 subfsp. *tranquillus, Palmae* (Plate 4.3., figs. 1-4)

Remark. - This fossil pollen grain was compared to recent taxa by KEDVES and BOHONY (1966) to establish the nearer botanical affinity. SEM pictures were published by KEDVES (1979) and from specimens of the Middle Eocene layers of the Dorog coal basin.

The tectate perforate ectexine is well illustrated in picture 1 and 3 of Plate 4.3. The tangential section of the infratectal layer represents well the columellar character of this middle ectexine layer. Two layers may be distinguished on the highly magnified picture (Plate 4.3., fig. 4) of the foot layer. The inner one may be endexine with degraded lamellar system. Granular units are also present in this part, which represents the larger molecular structures. Worth mentioning is that the embedding organic material is not closely connected to the tectum.

Cupuliferoidaepollenites quisqualis (POTONIÉ 1934) POTONIÉ 1960, *Fagaceae* or *Leguminosae* (Plate 4.4., figs. 1,2)

Three blocks (85/64, 85/66 and 86/4) were treated during 2.5 hours, another two (85/37 and 85/40) during 5 hours. Partial degradation was observed at the last mentioned experiment we present one example from these pollen grains (Plate 4.4., figs. 1,2).

The surface of these pollen grains is covered with the organic material. This may be a more or less amorphous layer with the thickness of the ectexine. The desintegration of the ectexine is well shown (Plate 4.4., figs. 1,2) in particular it is the infratectal layer which is sometimes completely destroyed (Plate 4.4., figs. 1,2).

Cupuliferoidaepollenites liblarensis (THOMSON, in POTONIÉ, THOMSON et THIERGART, 1950), POTONIÉ 1960, *Fagaceae* or *Leguminosae* (Plate 4.5., figs. 1-4)

Five blocks (85/41, 85/49, 85/60, 86/2 and 86/6). The pollen grains embedded into the blocks no: 85/49, 85/60 and 86/6 were treated during 2.5 hours, the other two; 85/41, 86/2, during 5 hours. As the best example we present the results of the 86/2 block.

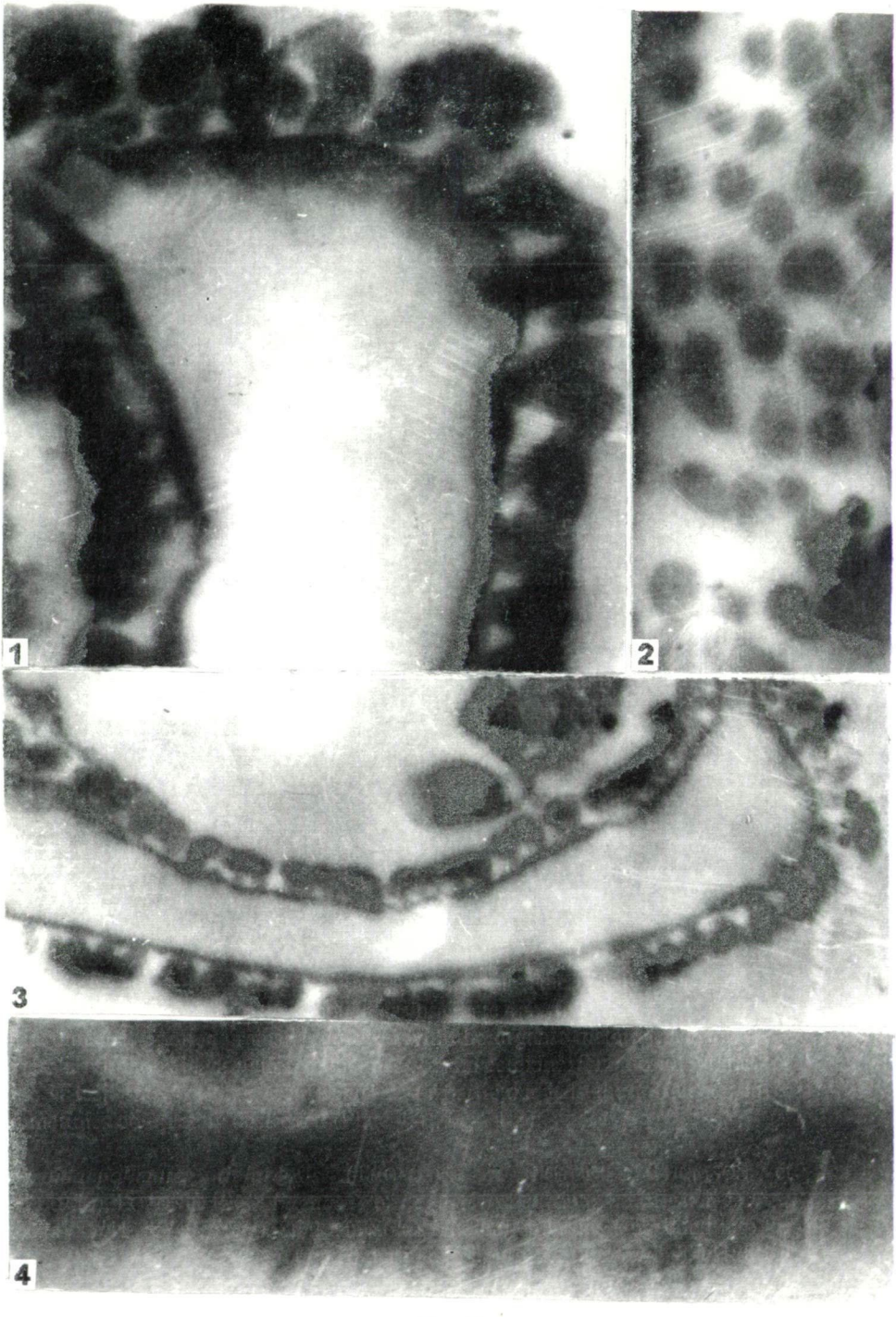


Plate 4.3.

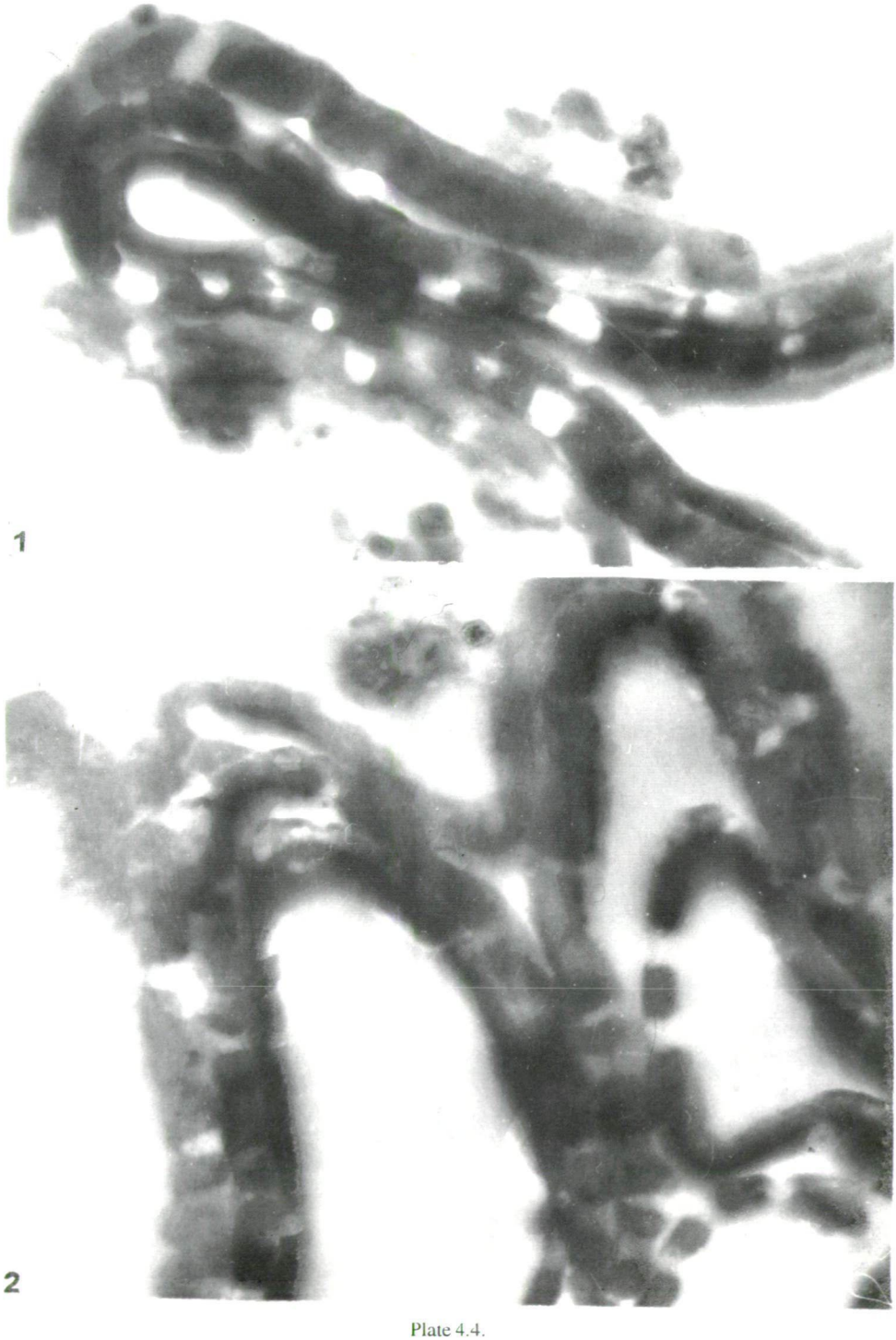


Plate 4.4.

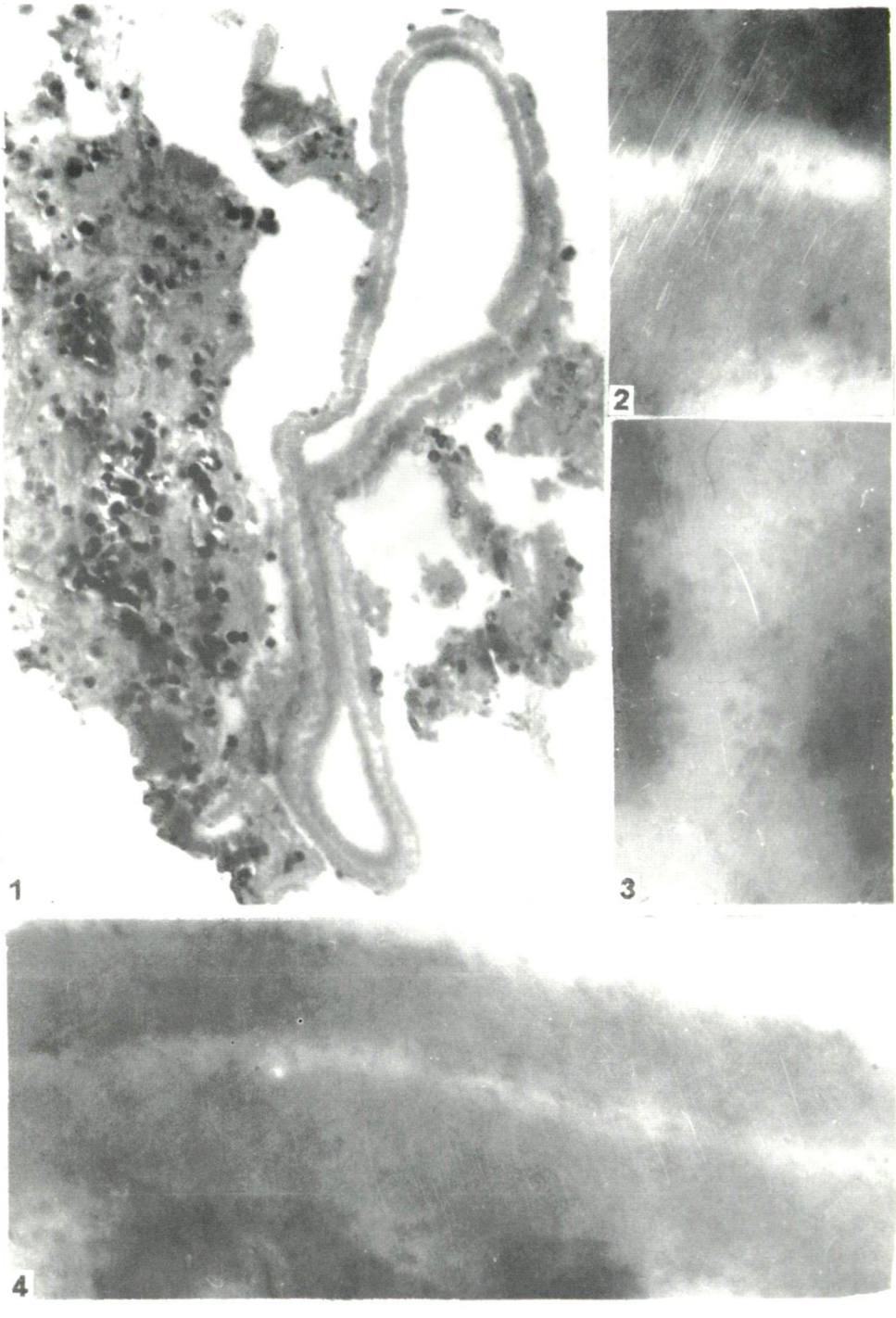


Plate 4.5.

Plate 4.3.

1-4. *Monocolpopollenites tranquillus* (POTONIÉ 1934) THOMSON et PFLUG 1953 subfsp. *tranquillus, Palmae*, block number: 85/51.
1. Negative no: 5456, 50.000x.
2. Negative no: 5458, 64.000x.
3. Negative no: 5455, 20.000x.
4. Negative no: 5463, 250.000x.

Plate 4.4.

1,2. *Cupuliferoidaepollenites quisqualis* (POTONIÉ 1934) POTONIÉ 1960, *Fagaceae* or *Leguminosae*, block number: 85/40.
1. Negative no: 5277, 50.000x.
2. Negative no: 5279, 50.000x.

Plate 4.5.

1-4. *Cupuliferoidaepollenites liblarensis* (THOMSON, in POTONIÉ, THOMSON et THIERGART, 1950) POTONIÉ 1960, *Fagaceae* or *Leguminosae*, block number: 86/2.
1. Negative no: 5679, 15.000x.
2. Negative no: 5682, 150.000x.
3. Negative no: 5682, 150.000x.
4. Negative no: 5685, 150.000x.

The surface of the pollen grains is completely covered with the spongy or lamellar organic material. There are several electron dense globular particles in the embedding material. The ultrastructure of this kind of embedding organic matter is similar to those published by LUGARDON, RAYNAUD and HUSSON (1991); Planche I, fig. 6.: "Part of large mass of dense, heterogeneous MAT with a cluster of packed laminae (arrowhead) and scattered ovoid elements (arrows). Upper Cretaceous, Iran. x50.000." The degradation of the infratectal layer is well shown on the general survey picture (Plate 4.5., fig. 1) too. The more or less completely disparition of the infratectal layer is illustrated on the highly magnified pictures (Plate 4.5., figs. 2-4). There are electron dense granules in the inner part of the tectum and the outer part of the foot layer which are in the larger sporopollenin globular dimension. The innest part of the exine is electron dense, this is in all probability the endexine (Plate 4.5., figs. 1 and 4). In picture 4 of the Plate 4.5., the desintegration of the tectum is illustrated.

Cupuliferoipollenites insleyanus (TRAVERSE 1955) POTONIÉ 1960 *Fagaceae, Castanea* (Plate 4.6., figs. 1,2)

The surface of the investigated pollen grain was rarely covered with the organic embedding material with electron dense granular particles. Fig. 1 in Plate 4.6., illustrate the degradation in the apertural area. Strong degradation of the infratectal layer was also observed in particular in the highly magnified picture (Plate 4.6., fig. 2). Less characteristic molecular structures were also observed.

Cupuliferoipollenites oviformis (POTONIÉ 1931) POTONIÉ 1960, *Fagaceae, Castanea* (Plate 4.7., figs. 1-5)

Pollen grains of two blocks (85/35 and 85/55) were investigated. The general survey pictures (Plate 2.7., figs. 1,4) illustrate well the characteristic ectexine of this kind of pollen grain, namely the thick tectum and foot layer and the relatively thin infratectal

layer. The embedding organic material is not always connected to the surface of the tectum. The characteristic lamellar ultrastructure of this material and the electron dense globular particles are well shown in fig. 5 of Plate 4.7., cf. LUGARDON, RAYNAUD and HUSSON (1991). The ectexine is resistant not so characteristic degradation was established at the ultrastructure of the infratectal layer.

Cupuliferoipollenites pusillus (POTONIÉ 1934) POTONIÉ 1960, *Fagaceae,* cf. *Castanea.* (Plate 4.8., figs. 1-3)

Pollen grains of the following blocks were partially dissolved during 2.5 hours: 85/31, 85/56, 85/57, 85/58, and one during 5 hours; 85/38. The exine ultrastructure is similar to the previous species including the interesting lamellar embedding organic material (Plate 4.8., fig. 2). The thin columellar infratectal layer more or less preserved. Beneath the foot layer the electron dense endexine is illustrated in picture 3, Plate 4.8. In several places the endexine is completely destroyed, and the inner surface of the foot layer is partially degraded and the globular larger molecular structures are well shown; Plate 4.8., fig. 1.

Retitricolpites thomsonii KEDVES 1982, in KEDVES and RUSSELL (Plate 4.9., figs. 1-3, plate 4.10., figs. 1,2, plate 4.11., figs. 1-3)

Pollen grains investigated from the blocks 85/62 and 86/1 were dissolved during 2.5 hours, 85/40, 85/42 and 86/3 during 5 hours. The characteristic exine ultrastructure of this exine is well shown in pictures no 1 in Plate 4.9. and 4.10. There are tangential sections also from the retipilate tectum (Plate 4.10., fig. 1) and from the columellar infratectal layer (Plate 4.1., fig. 1). The lamellar embedding organic material is in several places connected to the surface of the tectum. The electron dense granular particles are in the holes of the infratectal layer (Plate 4.10., fig. 2), cf. LUGARDON, RAYNAUD and HUSSON (1991). In general the exine of this kind of pollen grains is resistant, but degradation was observed in the infratectal layer (Plate 4.9., fig. 3).

In the apertural area the lamellar endexine is also degraded (Plate 4.9., fig. 3).

Brevaxones
Normapolles

Stephanoporopollenites hexaradiatus (THIERGART 1940) THOMSON et PFLUG 1953 cf. *tribinae* KRUTZSCH 1961 (Plate 4.12., figs. 1-3)

The investigated specimen was extremely damaged. The infratectal layer disappeared in consequence of the sedimentation or the experimental processes (Plate 4.12., figs. 1-3). The tectum is thinner than the foot layer, light, partial degradation zones were observed. The lower layer separate sometimes. The embedding organic material is not closely connected to the tectum, its ultrastructure is lamellar, with globular electron dense particles.

Plicapollis pseudoexcelsus (KRUTZSCH 1958) KRUTZSCH 1961 subfsp. *pseudoexcelsus* (Plate 4.13., figs. 1-3)

The lamellar embedding organic material is in general connected to the tectum. Worth mentioning is that in this case electron dense granular particles were not observed. The granular infratectal layer was completely destroyed. This is very characteristic at the annuli. Beneath the foot layer another electron dense layer is present with doubtful origin. This may be an inner embedding organic material or a peculiar inner

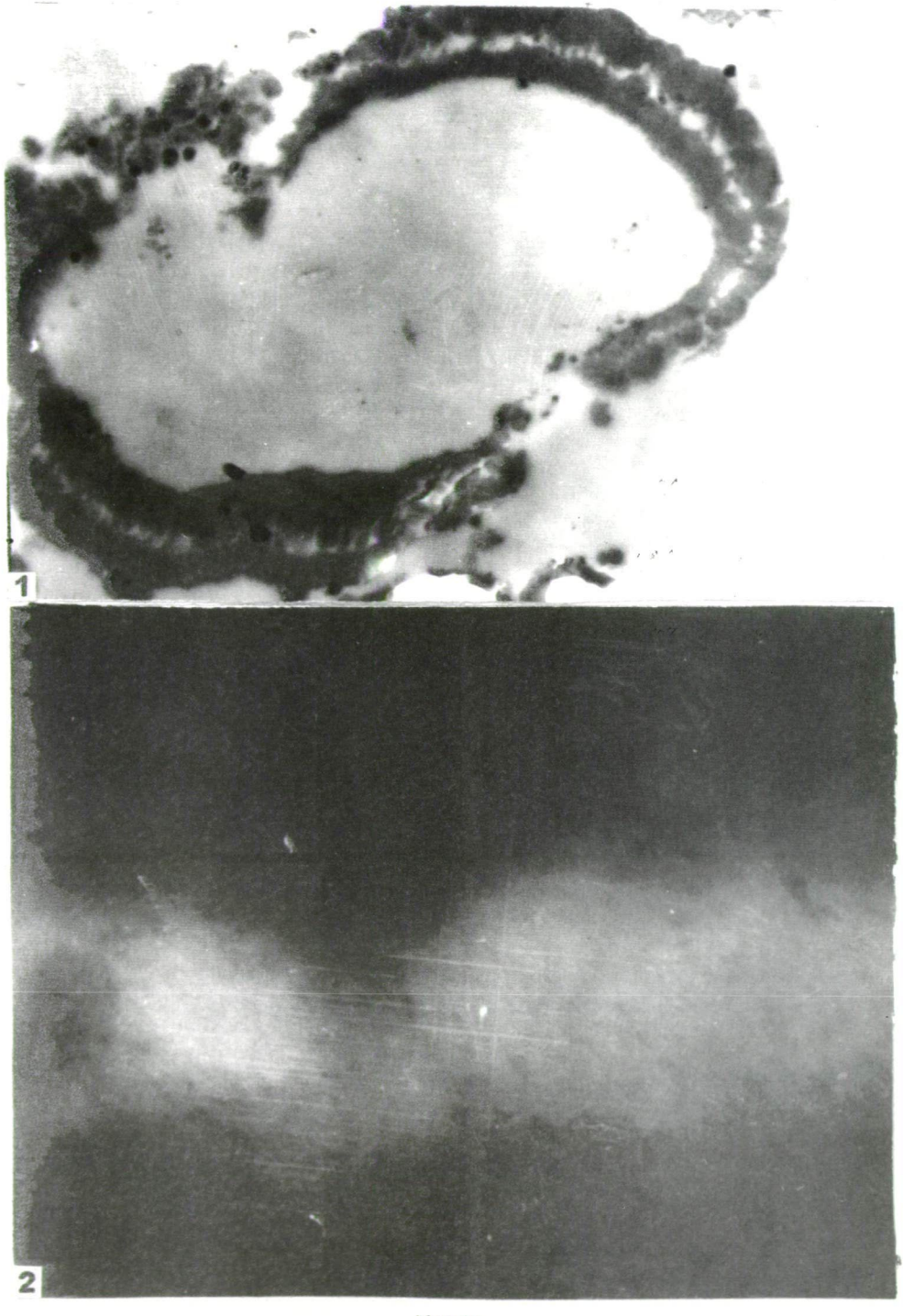
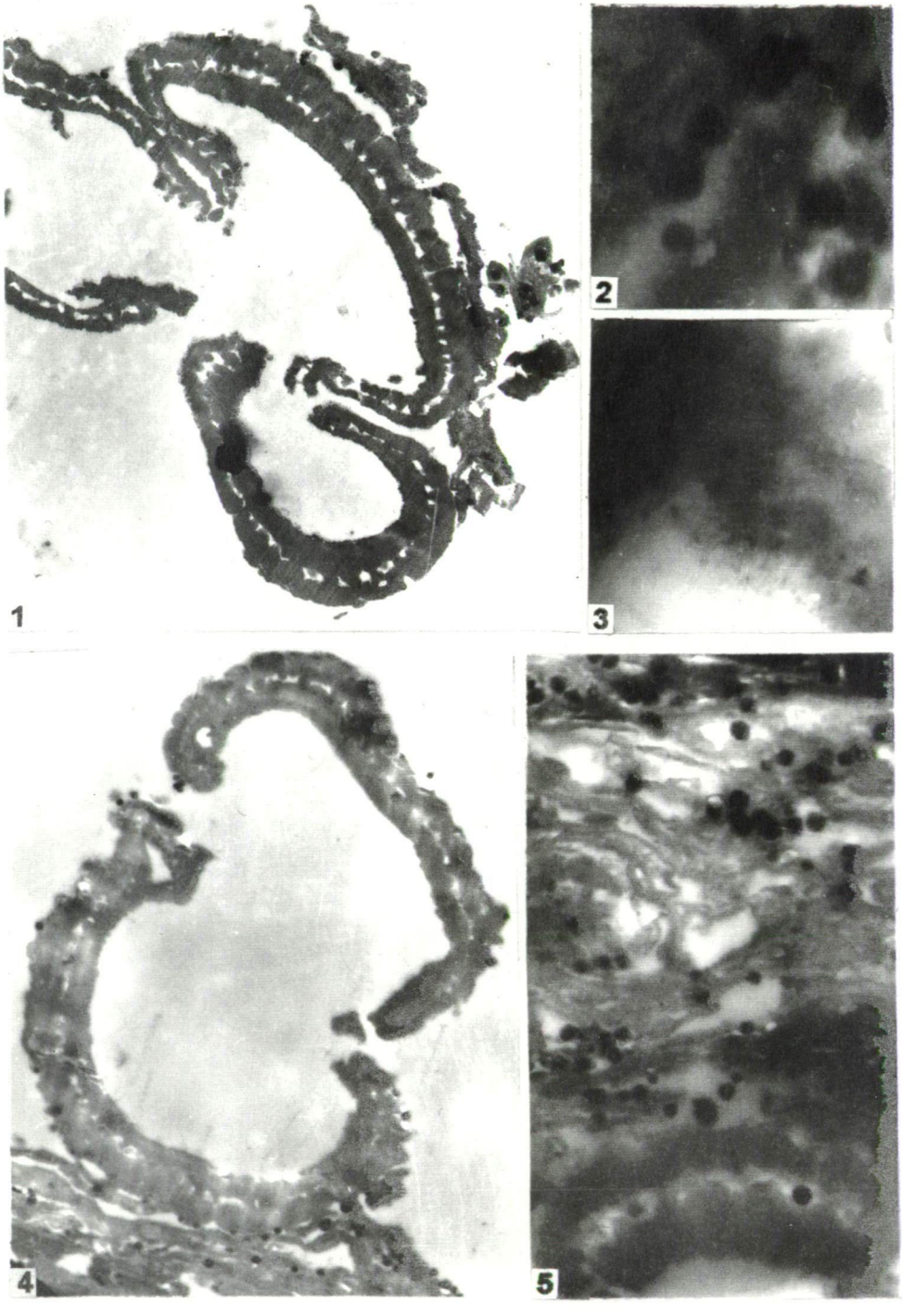


Plate 4.6.



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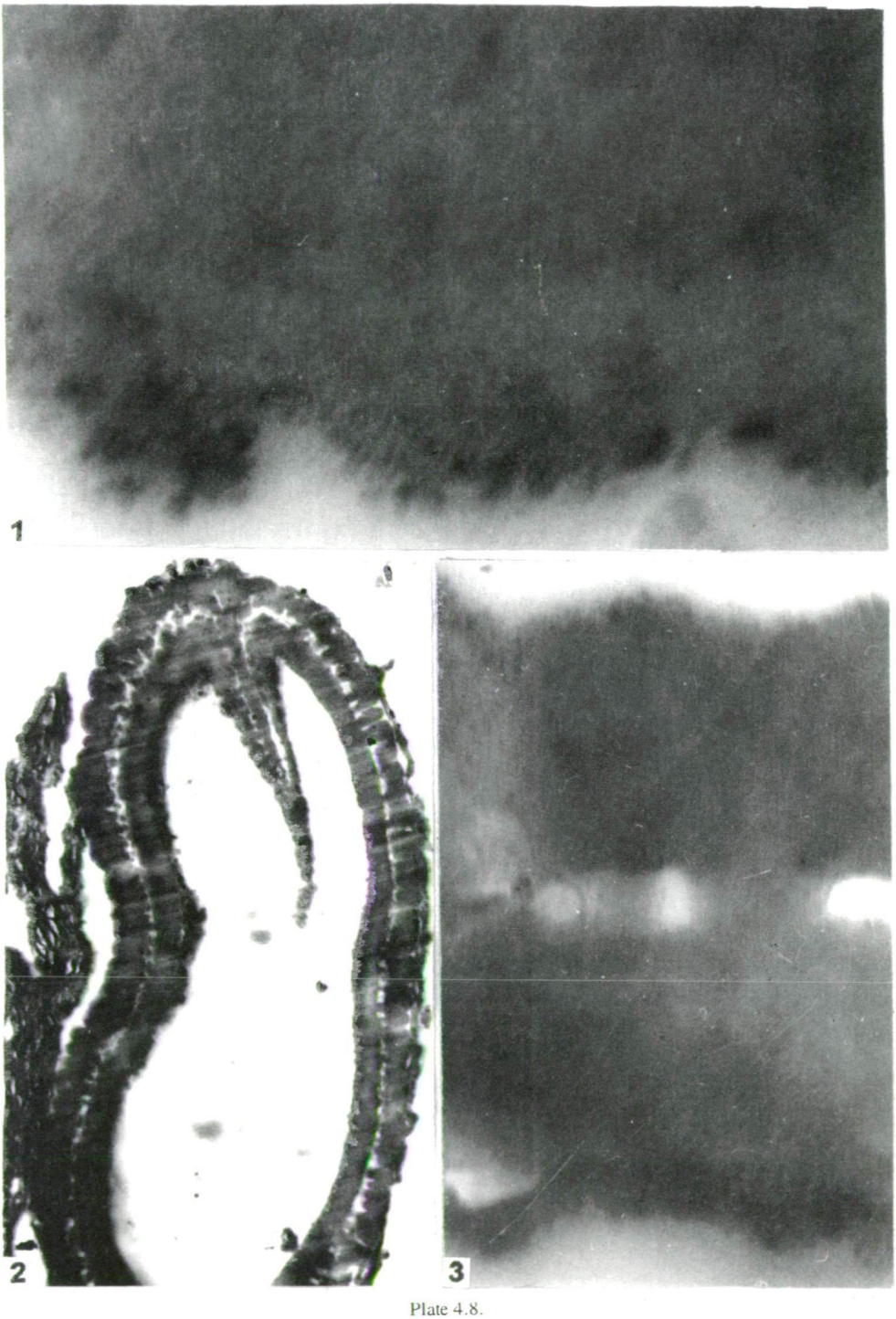

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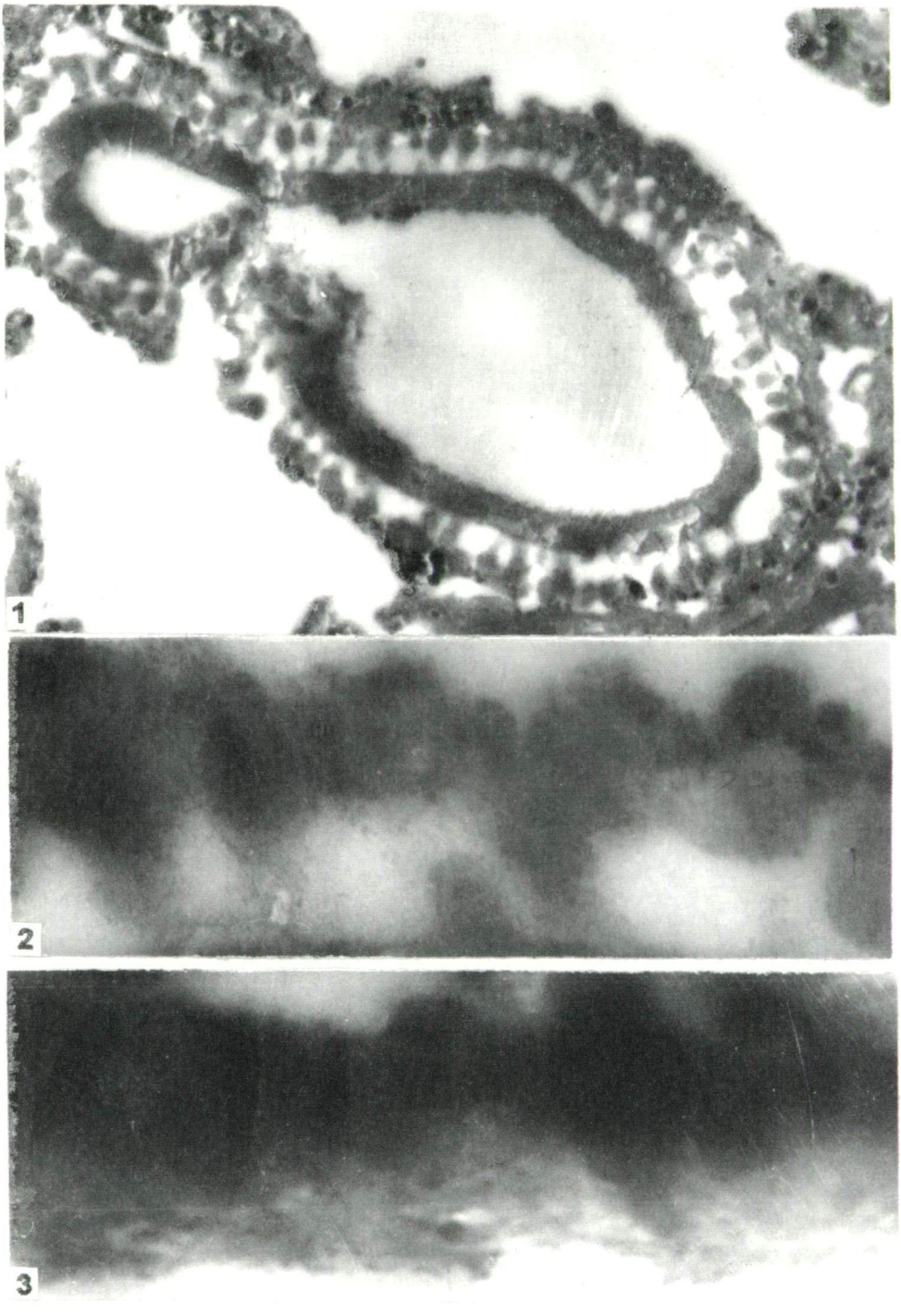

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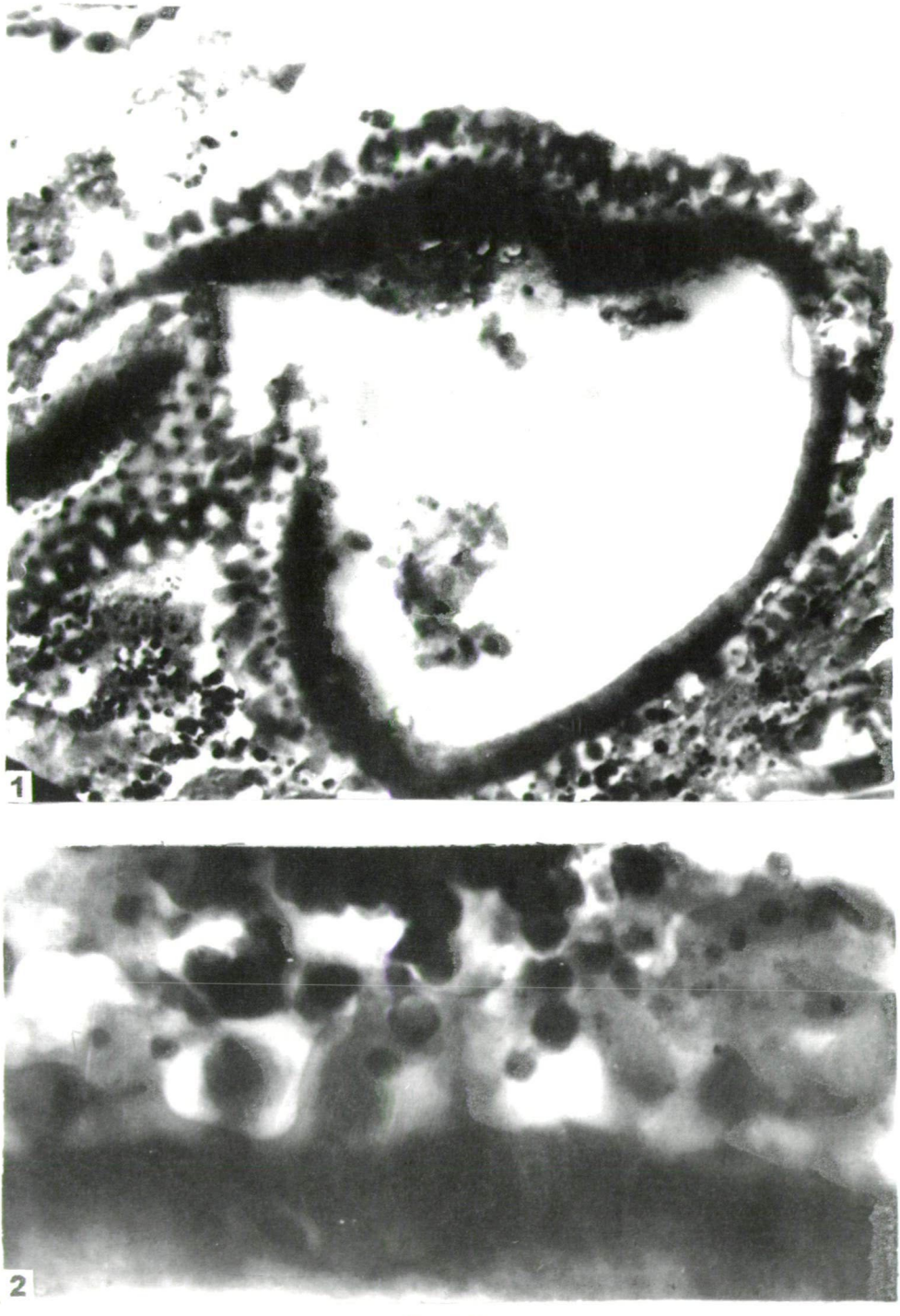

Plate 4.10.

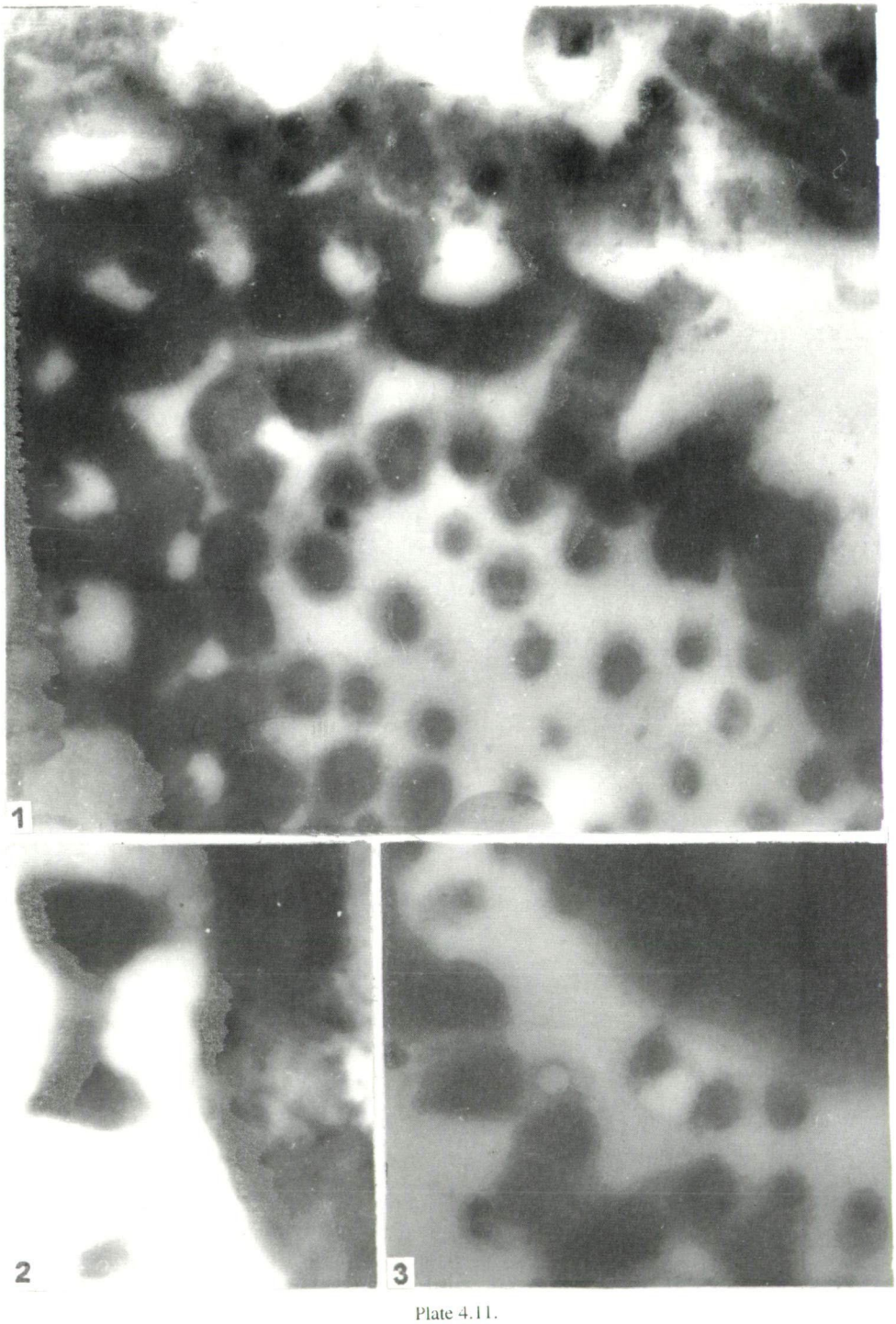

Plate 4.11.

Plate 4.6.

1,2. *Cupuliferoipollenites insleyanus* (TRAVERSE 1955) POTONIÉ 1960, *Fagaceae, Castanea*, block number: 85/63.
1. Negative no: 5615, 15.000x.
2. Negative no: 5619, 200.000x.

Plate 4.7.

1-5. *Cupuliferoipollenites oviformis* (POTONIÉ 1931) POTONIÉ 1960, *Fagaceae, Castanea*,
1. Block number: 85/35, negative no: 5213, 10.000x.
2-5. Block number: 85/55.
2. Negative no: 5487, 100.000x.
3. Negative no: 5487, 100.000x.
4. Negative no: 5477, 10.000x.
5. Negative no: 5486, 20.000x.

Plate 4.8.

1-3. *Cupuliferoipollenites pusillus* (POTONIÉ 1934) POTONIÉ 1960, *Fagaceae* cf. *Castanea*.
1. Block number: 85/31, negative no: 5208, 250.000x.
2. Block number: 85/48, negative no: 9275, 8.000x.
3. Block number: 85/56, negative no: 5488, 100.000x.

Plate 4.9.

1-3. *Retitricolpites thomsonii* KEDVES 1982, in KEDVES et RUSSELL.
1,2. Block number: 85/57.
1. Negative no: 5496, 15.000x.
2. Negative no: 5493, 100.000x.
3. Block number: 85/57, negative no: 5609, 100.000x.

Plate 4.10.

1,2. *Retitricolpites thomsonii* KEDVES 1982, in KEDVES et RUSSELL, block number: 86/3.
1. Negative no: 5686, 10.000x.
2. Negative no: 5692, 50.000x.

Plate 4.11.

1-3. *Retitricolpites thomsonii* KEDVES 1982, in KEDVES et RUSSELL, block number: 85/40.
1. Negative no: 5283, 50.000x.
2. Negative no: 5281, 50.000x.
3. Negative no: 5285, 48.000x.

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part of the foot layer. In this case it is interesting, that it is a hiatus between the foot layer and the above mentioned layer.

Postnormapolles

Platycaryapollenites platycaryoides (ROCHE 1969) KEDVES 1992, *Juglandaceae, Platycarya* (Plate 4.14., figs. 1-3)

The investigated pollen grain is not completely closed in the lamellar embedding material (GLIKSON and TAYLOR 1986). The electron dense granular particles are sometimes on the outer surface of the tectum, or on the inner surface of the foot layer. The infratectal layer is granular, which are well preserved. Sometimes larger molecular structures were observed near the electron dense particles of the embedding material.

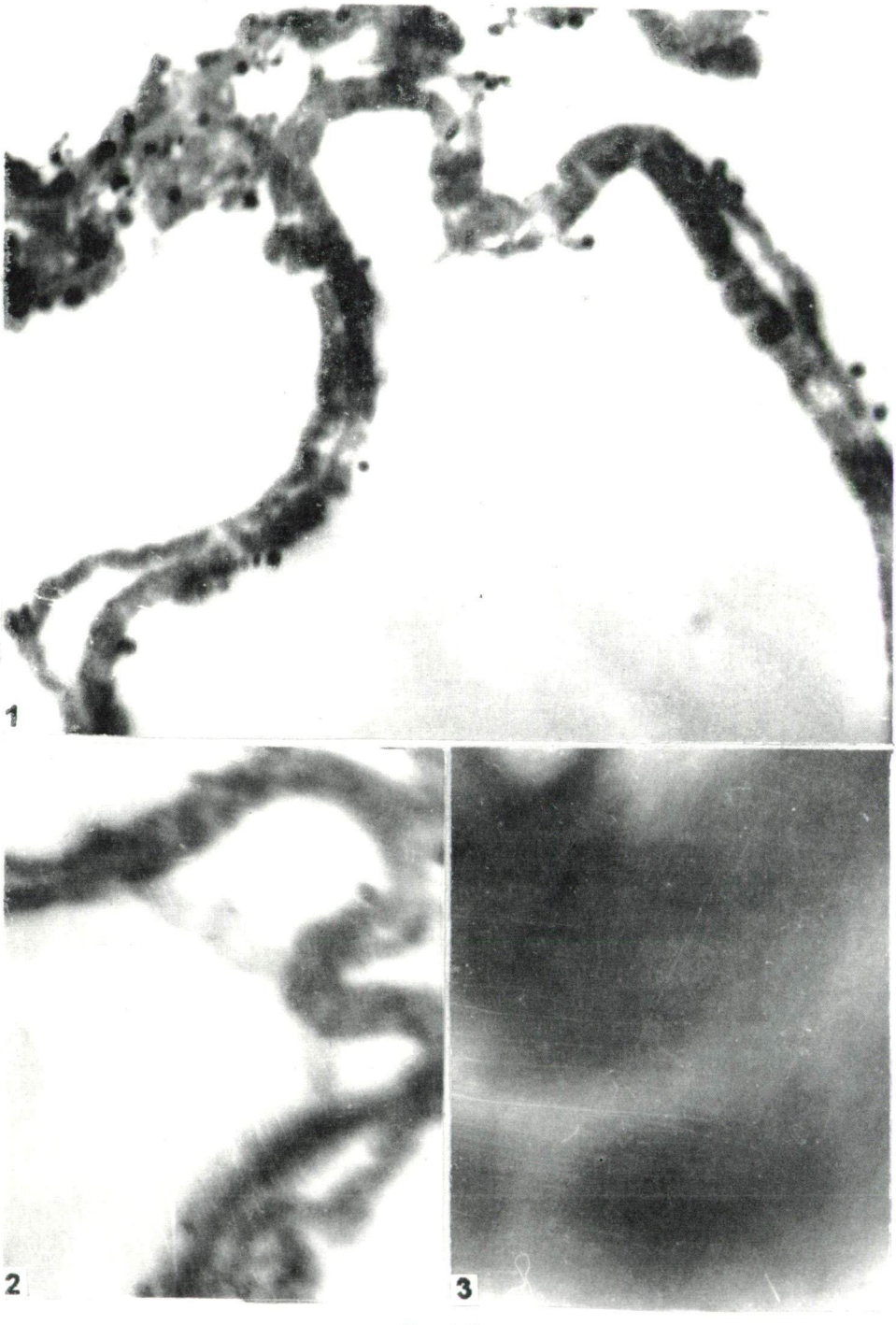


Plate 4.12.

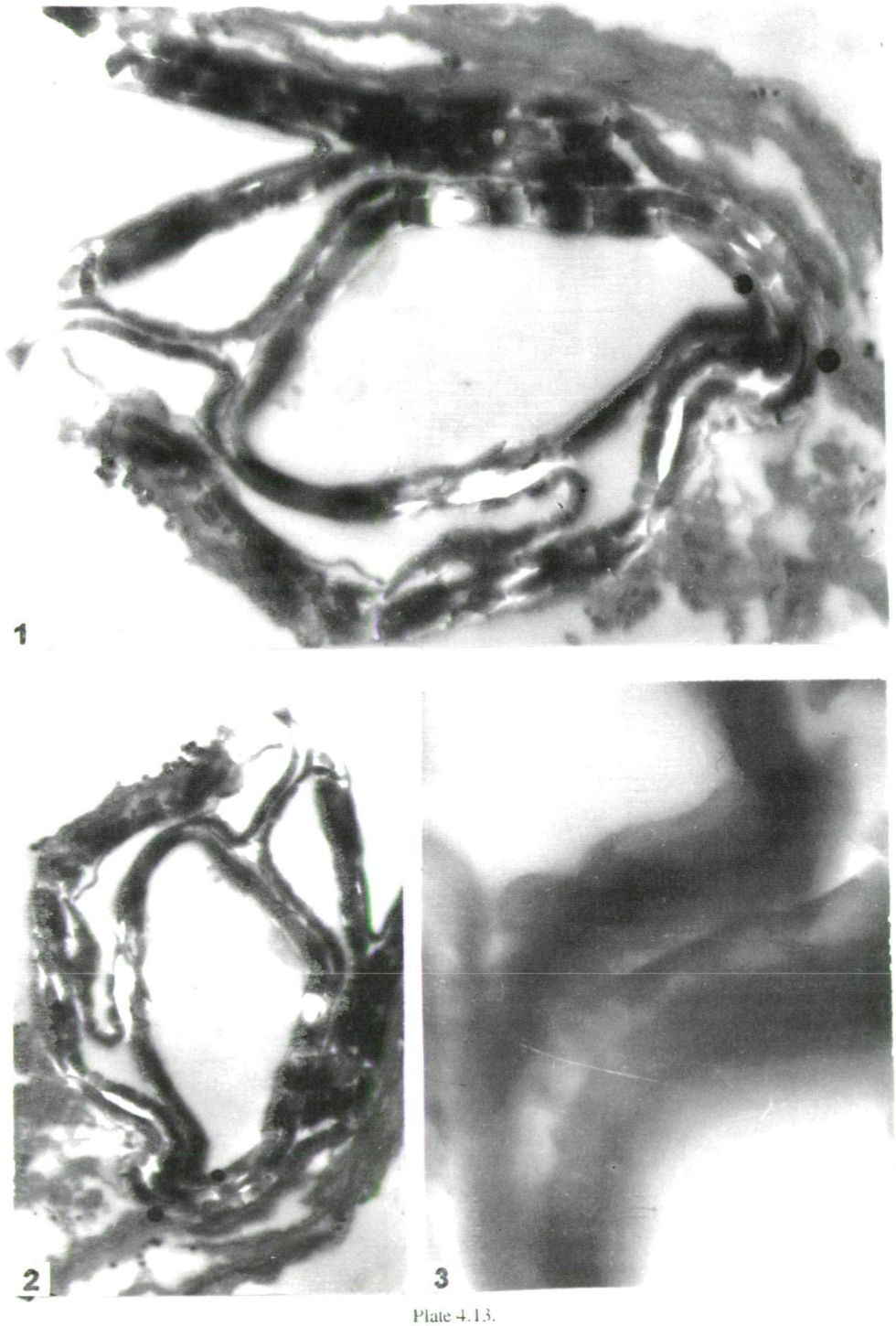
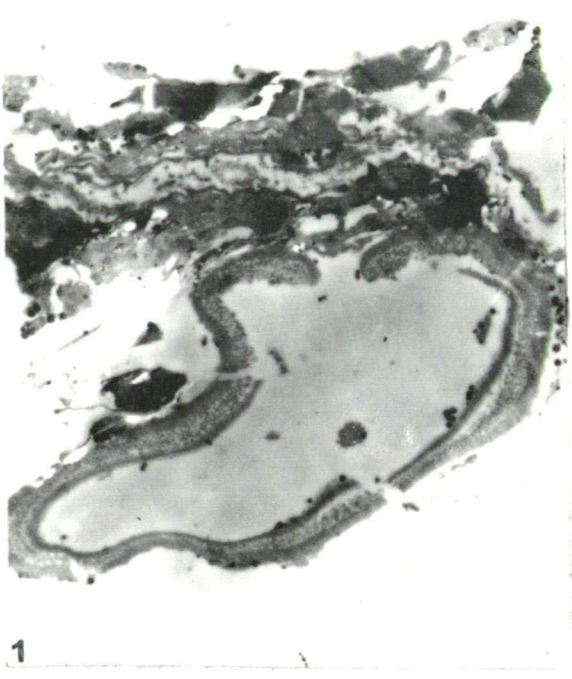
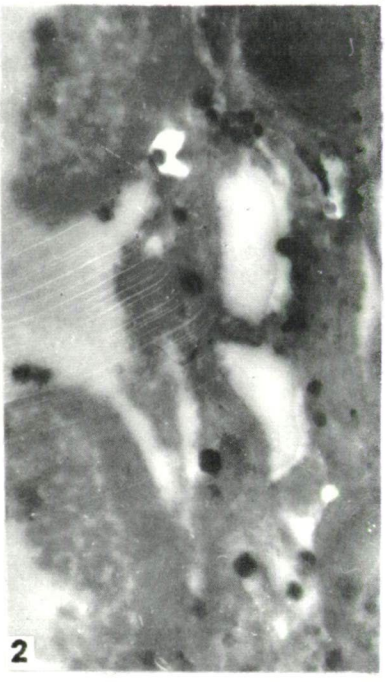


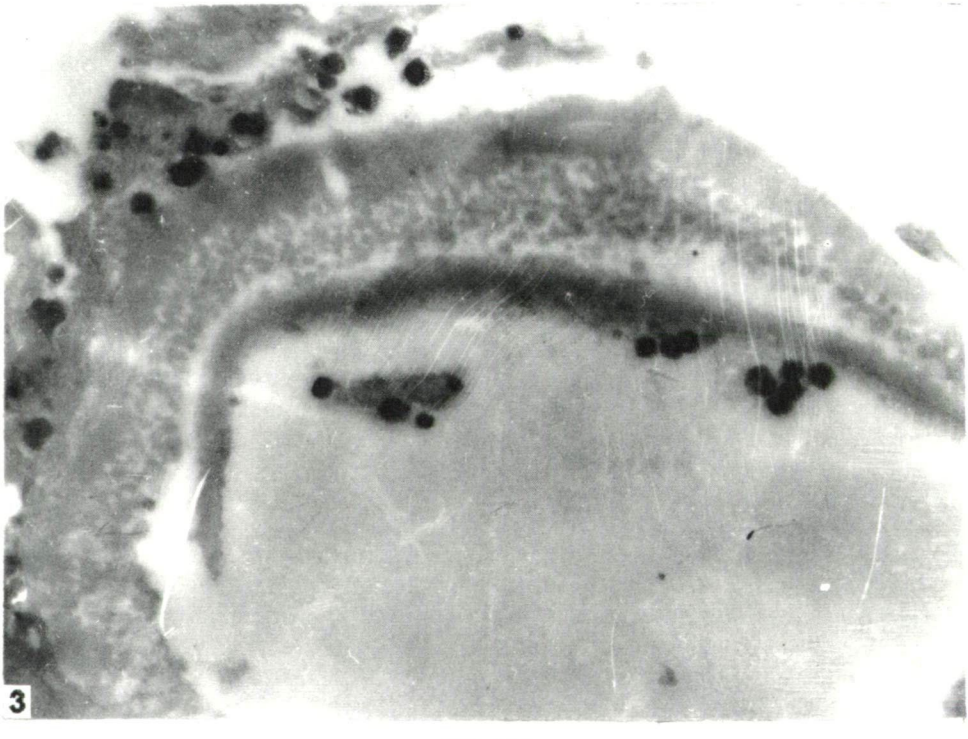
Plate 4.13.



1



2



3

Plate 4.14.

Plate 4.12.

1-3. *Stephanoporopollenites hexaradiatus* (THIERGART 1940) THOMSON et PFLUG 1953 cf. *tribinae* KRUTZSCH 1961, block number: 85/61.
1. Negative no: 5587, 25.000x.
2. Negative no: 5581, 25.000x.
3. Negative no: 5582, 250.000x.

Plate 4.13.

1-3. *Plicapollis pseudoexcelsus* (KRUTZSCH 1958) KRUTZSCH 1961 subfsp. *pseudoexcelsus*, block number: 85/47.
1. Negative no: 5336, 5.000x.
2. Negative no: 5336, 2.500x.
3. Negative no: 5338, 100.000x.

Plate 4.14.

1-3. *Platycaryapollenites platycaryoides* (ROCHE 1969) KEDVES 1992, *Juglandaceae, Platycarya*, block number: 85/65.
1. Negative no: 5643, 5.000x.
2. Negative no: 5646, 25.000x.
3. Negative no: 5645, 25.000x.

Discussion and Conclusions

1. The molecular system of the investigated saccate *gymnosperm* pollen grains is very resistant. After the partial dissolution of the recent saccate *gymnosperm* pollen grains of *Pinus sylvestris* KEDVES, HORVÁTH, BORBOLA and TÓTH (1999) emphasized the same.

2. *Angiosperm* pollen grains

2.1. At the *angiosperm* pollen grains resistant tectum and the foot layer was observed in particular at the *Cupuliferoipollenites* fspp. KEDVES et al (1998) established, that the exine of *Castanea sativa* is resistant to the organic solvents, thinnings of the exine and protrusions in the apertural area were observed only.

2.2. Partial degradation of the *angiosperm* exines.

2.2.1. Degradation of the tectum is relatively rare (cf. *Stephanoporopollenites*).

2.2.2. Alterations in the ultrastructure of the infratectal layer.

Completely destroyed at *C. quisqualis* and *C. liblarensis* and at the two investigated *Normapolles* taxa. Worth mentioning is that the granular infratectal layer of the *myricaceous Plicapollis pseudoexcelsus* destroyed completely, and the also granular infratectal layer of *Platycaryapollenites* is very resistant. To this KEDVES, KÁROSSY and BORBOLA (1997) pointed out as follows, p. 54: "The resistance of the pollen grains of *Platycarya strobilacea* is also interesting."

Partial degradation was observed with larger biopolymer structures at *C. insleyanus* and *Retitricolpites thomsonii.*

Secondary lamellar ultrastructure appeared with molecular structures at the foot layer of *Monocolpopollenites tranquillus.* Larger globular molecular units at *Retitricolpites thomsonii* and at the inner surface of the foot layer of *Platycaryapollenites.*

3. The organic embedding material is also another subject for investigation. Till this time the following types may be established:

3.1. Lamellar structures without electron dense granular particles. This is not very often.

3.2. Lamellar structures with electron dense globular particles. According to GLIKSON and TAYLOR (1986) for the origin to these particles the microbial function may be presumed. To this the investigated *Platycaryapollenites* serve documents. Namely these granules are on the surface of the tectum or on the inner surface of the foot layer. The larger molecular structures were discovered near the electron dense particles.

3.3. Interesting and not so often when the embedding organic material is completely close to the surface of the pollen grains seemingly forming another outer layer of the exine. This may be in connection to the electrostatic charge of the surface of the pollen grain.

Finally, based on these present results it is necessary to continue further, stronger partial degradation experiment on this material.

Acknowledgements

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References

EHRLICH, H.G. and HALL, J.W. (1959): The ultrastructure of Eocene pollen grains. – Grana Palynologica *2*, 32-35.

ELSIK, W.C. (1968): Palynology of a Paleocene Rockdale lignite, Milam County, Texas I. - Morphology and Taxonomy (End.) - Pollen et Spores *10*, 263-314.

GLIKSON, M. and TAYLOR, G.H. (1986): Cyanobacterial mats: major contributors to the organic matter in Toolebuc Formation oil shales. - Geol. Soc. of Australia Special Publication *12*, 273-286.

KEDVES, M. (1965): A new *Restionaceae* pollen type from the Hungarian Lower Tertiary layers. - Advancing Frontiers of Plant Sciences *13*, 49-54.

KEDVES, M. (1979): Scanning electron microscopical investigations into the sporomorphs of the coal layers in the Dorog Basin. - Acta Biol. Szeged. *25*, 35-44.

KEDVES, M. (1986): Dégradation expérimentale de la paroi pollinique. - VI Simposio de Palinologia, Programa Resumenes de las Comunicaciones (Orales y Poster), Salamanca, 24-26 Septiembre, 20.

KEDVES, M. (1988): Dégradation expérimentales de la paroi pollinique. - Actas de Palinologia (Actas del VI Simposio de Palinologia, A.P.L.E.) (eds.: J. CIVIS LLOVERA y Ma, F. VALLE HERNÁNDEZ), 395-408.

KEDVES, M. (1990): Transmission electron microscopy of the fossil angiosperm exines. - Szeged.

KEDVES, M. (1992): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne. VII. - Acta Biol. Szeged. *38*, 33-46.

KEDVES, M. (1994): Transmission electron microscopy of the fossil *gymnosperm* exines. - Szeged.

KEDVES, M. et BOHONY, E. (1966): Observations sur quelques pollens de palmiers provenant des couches tertiaires de Hongrie. - Pollen et Spores *8*, 141-147.

KEDVES, M., HORVÁTH, E., BORBOLA, A. and TÓTH, A. (1999): LM investigations of partially dissolved sporomorphs III. - Plant Cell Biology and Development (Szeged) *10*, 75-82.

KEDVES, M., HORVÁTH, E., MÉSZÁROS, E., MÉSZÁROS, R., RONTÓ, G., SZLÁVIK, N., GAUDÉNYI, SZ. and KALMÁR, Á. (1998): LM investigations of partially dissolved sporomorphs II. - Plant Cell Biology and Development (Szeged) *9*, 76-87.

KEDVES, M., KÁROSSY, Á. and BORBOLA, A. (1997): LM investigations of partially dissolved sporomorphs I. - Plant Cell Biology and Development (Szeged) *8*, 44-55.

KEDVES, M. and RUSSELL, D.A. (1982): Palynology of the Thanetian layers of Menat. - Palaeontographica *B, 183*, 87-150.

KEDVES, M., STANLEY, E.A. and ROJIK, I. (1974): Observations nouvelles sur l'ectexine des pollens fossiles des *Angiospermes* de l'Eocène inférieur. - Pollen et Spores *16*, 425-427.

KRUTZSCH, W. (1958): Sporen- und Pollengruppen aus der Oberkreide und dem Tertiär Mitteleuropas und ihre stratigraphische Verteilung. - Z. angew. Geol. *3*, 509-548.

KRUTZSCH, W. (1960): Über *Thomsonipollis magnificus* (TH. & PF.) n. fgen. n. comb. und Bemerkungen zur regionalen Verbreitung einiger Pollengruppen im älteren Paläogen. - Freiberger Forschungshefte *86*, 54-65.

KRUTZSCH, W. (1961): Beiträg zur Sporenpaläontologie der präoberoligizänen kontinentalen und marinen Tertiärablagerungen Brandenburgs. - Berichte der Geol. Ges. *4*, 290-343.

KRUTZSCH, W. (1971): Atlas der mittel- und jungtertiären dispersen Sporen- und Pollen- sowie der Mikroplanktonformen des nördlichen Mitteleuropas. Lief. VI. (Saccites und "Inaperturates"). - VEB Gustav Fischer Verlag, Jena.

LUGARDON, B., RAYNAUD, J.-F. et HUSSON, P. (1991): Données ultrastructurales sur la matière organique amorphe des kerogènes. - Palynosciences *1*, 69-88.

PFLUG, H.D. und THOMSON, P.W. (1953): In: THOMSON und PFLUG.

POTONIÉ, R. (1931): Pollenformen aus tertiaeren Braunkohlen (3. Mitt.). - Jb. Preuss. Geol. L.A. f. 1931, *52*, 1-7.

POTONIÉ, R. (1934): Zur Mikrobotanik des eozänes Humodils des Geiseltales. - Arb. aus Inst. Paläobotanik u. Petrogr. Brenngesteine *4*, 25-125.

POTONIÉ, R. (1960): Synopsis der Gattungen der Sporae dispersae. III. - Beih. Geol. Jb. *39*, 1-189.

POTONIÉ, R., THOMSON. P.W. und THIERGART, F. (1950): Zur Nomenklatur und Klassifikation der Sporomorphae (Pollen und Sporen). (Zur Geologie der rheinischen Braunkohle 1). - Geol. Jb. f. 1949, *65*, 35-70.

ROCHE, E. (1969): Étude palynologique de sédiments du Montien continental et du Landénien supérieur en Hainaut. - Bull. Soc. belge Geol. Paleont. Hydrol. *78*, 131-146.

ROWLEY, J.R., ROWLEY, J.S. and SKVARLA, J.J. (1990): Corroded exines from Havinga's leaf mold experiment. - Palynology (USA) *14*, 53-79.

ROWLEY, J.R. and SRIVASTAVA, S.K. (1986): Fine structure of *Classopollis* exines. - Can. J. Bot. *64*, 3059-3074.

RUSSELL, D.E. (1982): The Geology of the Menat Basin, France. - Palaeontographica *B, 182*, 89,90.

SITTLER, C. et SCHULER, M. (1991): Une méthode d'analyse quantitative absolue de la fraction organique constituant le palynfacies d'une roche sedimentaire. - Palynosciences *1*, 59-68.

SKARBY, A., ROWLEY, J.R. and NILSSON, L. (1990): Exine structure of Upper Cretaceous *Normapolles* grains from anthers (Northeastern Scania, Sweden). – Palynology *14*, 145-473.

THIERGART, F. (1940): Die Mikropaläontologie als pollenanalyse im Dienste der Braunkohlenforschung. - Schr. a.d. Gebiet der Brennstoffgeol. *13*, 1-82.

THOMSON, P.W. (1950): In POTONIÉ, R., THOMSON, P.W. und THIERGART, F.

THOMSON, P.W. und PFLUG, H.D. (1953): Pollen und Sporen des mitteleuropäischen Tertiärs. - Palaeontographica *B, 94*, 1-138.

TRAVERSE, A. (1955): Pollen analysis of the Brandon lignite of Vermont. - Bureau of Mines of Investigations *5151*, 1-107.

5. LM, SEM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED BOTRYOCOCCUS BRAUNII KÜTZ. COLONIES FROM HUNGARIAN UPPER TERTIARY OIL SHALE I.

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Abstract

During our newest experimental investigations on the *Botryococcus braunii* colonies isolated from Upper Pannonian oil shale from Pula nine kinds of experiments were carried out. For the partial degradation of the colonies 2-aminoethanol, $KMnO_4$, and merkaptoethanol were used. The results obtained by the different methods were compared. Particular attention was paid for the different organization levels of the biopolymer units of the wall of the colonies. The problems of the different methods are discussed.

Key words: Alginite, partial degradation, LM, SEM, TEM.

Introduction

The hydrocarbon producing colonies of the *Botryococcus* KÜTZING genus are very interesting and peculiar among the *Algae*. This was the reason that the taxonomic position was not always unanimous. According to FRITSCH (1935) and KISS (1939) this genus belongs to *Xanthophyceae, Heterochloridales*. WILLE (in ENGLER and PRANTL 1910) classed into the *Chlorophyta (Chlorococcales - Protococcales) Botryococcaceae*, PANKOW (1976) into the *Chlorococcales, Dictyosphaeraceae*. There are further comprehensive papers in this respect, e.g.: BATTEN and GRENFELL (1996). The most important papers concerning the basic morphology of these peculiar colonies are as follows: BLACKBURN and TEMPERLEY (1936), FRÉMY and DANGEARD (1938), CHADEFAUD and EMBERGER (1960), COMBAZ (in DURAND, 1980), LARGEAU, DERENNE, CLAIRAY et al. (1990), GLIKSON, LINDSAY and SAXBY (1989), VÉR (1994) and BATTEN and GRENFELL (1996). The most important morphological characteristic features may be summarized as follows: 1. The colonies are enveloped in mucilage (=Schleimmasse = mucilagineuse = enveloppe gelatineuse hyaline = hydrocarbon matrix). FRÉMY and DANGEARD (1938) established polymorphism at the *Botryococcus* colonies both living and fossil. E. NAGY (1969) emphasized also the problem of polymorphism what may be in consequence of the ontogenetical stages, ecologic factors, such as temperature, light intensity, water composition, pH, etc. 2. The cellular components of the colonies are pedunculus and cupula (cup). Two cupulae form one polipier following CHADEFAUD and EMBERGER (1960). 3. Developmental stages of *Botryococcus braunii*, both recent and fossil, were

published by GUY-OHLSON (1992). Autospores on the sides of the cups of young colonies were published by ERLSTRÖM, GUY-OHLSON and SIVHED (1994) from Jurassic-Cretaceous layers, using the SEM method. The organelles of the protoplasm were summarized by CHADEFAUD and EMBERGER (1960), and COMBAZ (1980). The LM wall structure based on TEMPERLEY (BLACKBURN and TEMPERLEY, 1936) and LARGEAU et al. (1990) was summarized by BATTEN and GRENFELL (1996) as follows: The cellulose wall is closed by the cell cap. The cellulose wall is surrounded by three cups, the outest is the first cup, the third cup is in contact with the cellulose wall. The first taxonomical problems of the oil bearing alga were summarized by NARAYANA RAO and MISRA (1949): "yellow bodies" BERTRAND and RENAULT (1892), after *Reinschia australis*, and *Pila*. Later BLACKBURN and TEMPERLEY (1936), HARRIS (1938), TRAVERSE (1955) and GRAY (1960) reviewed the morphology, the geologic and the geographic distribution of *Botryococcus*. The taxonomic problems of the genus *Botryococcus* and *Gloeocapsomorpha* were discussed by KOSANKE and MYERS (1986), COLLINSON et al. (1994), BATTEN and GRENFELL (1996) and WICANDER, FOSTER and REED (1996). They pointed out that the *Gloeocapsomorpha* may not be cogeneric with *Botryococcus*. The ecology of *Botryococcus braunii* KÜTZ. was summarized by VÉR (1994) as follows; p. 12:

"i. Occurrence in eutrophic fresh water or in humid soil (WILLE, in ENGLER and PRANTL, 1910) in Europe, North America and Africa. These colonies can also be present in salt lakes or in the water of marine lagoons.

ii. Stenotherm species after the paper of KISS (1939). The geological distribution of this kind of algue is extremely large. JARZEN (1978) writes the following, p. 32: '*Botryococcus* KÜTZING (Pl. 1, fig. 3) is a colonial green algae, whose colonies form irregular globose masses encasted in a heavy, often dark, cohered mucilage. TRAVERSE (1955) has reviewed the fossil occurrences of the genus and notes that the fossil record probably extends back at least to the Ordovician.' (Cf. NARAYANA RAO and MISRA, 1949).

'The hydrocarbon secreting alga *Botryococcus* has been identified in organic remains of sediments ranging from Precambrian to Recent,' (GLIKSON, LINDSAY and SAXBY, 1989, p. 595)."

The colonies which may form the oil shale (Alginite) layers and its importance in the kerogen was recognized very early. In this way the industrial importance of this kind of algal colonies intensive and exhaustive and multidisciplinary researches were carried out. Regarding the chemical composition the three cups are the most important. Previously the sporopollenin content was established. Later based on the new results several alterations were pointed out, such as the extreme chemical variability of the colony matrix by BRENNER (1998), and the terms PRB ("dominated by linear fatty monocarboxylic acids but also comprise a linear dicarboxylic acid and a "pseudo" isoprenoid acid and exhibit a substantial contribution of isoprenoid acids." By DERENNE, LARGEAU and CASADEVALL (1991, p. 597), botryococcene (isoprenoid hydrocarbons) respectively botryococcane were introduced. To this we cite the paper of BERKALOFF, CASADEVALL, LARGEAU, et al. (1983), LARGEAU, CASADEVALL, KADOURI and METZGER (1984), DERENNE, LARGEAU, CASADEVALL and BERKALOFF, (1989), DERENNE, LARGEAU, CASADEVALL and CONNAN (1988a,b) and TEMPLIER, DIESENDORF, LARGEAU and CASADEVALL (1992).

TEM pictures were published from untreated recent colonies of *Botryococcus braunii* by WOLF and COX (1981), BURNS (1982), KADOURI, DERENNE, LARGEAU, et al. (1988), DERENNE, LARGEAU, CASADEVALL and BERKALOFF (1989), TEMPLIER, LARGEAU, CASADEVALL and BERKALOFF (1992).

SEM data from untreated colonies by TEMPLIER, LARGEAU, CASADEVALL and BERKALOFF (1992), ERLSTRÖM and GUY-OHLSON (1994) and GUY-OHLSON and LINDSTRÖM (1994).

TEM results of fossil colonies without experiment were published by KEDVES (1983) and DERENNE, LARGEAU, HETÉNYI, et al. (1997). SEM data from Hungarian *Botryococcus* colonies were published by E. NAGY (1976), GUY-OHLSON and LINDQVIST (1990) from Cambrian-Ordovician age from Sweden, DERENNE, LARGEAU, HETÉNYI, et al. (1997) from the locality of Pula (Hungary). From Early Permian untreated *Botryococcus* colonies GUY-OHLSON (1992), and GUY-OHLSON and LINDSTRÖM (1994) published new data. Single, compound colonies and autospores were illustrated. The different stages of preservation were also discussed. Jurassic data from GUY-OHLSON (1996).

The presence of the *Botryococcus* colonies in the Hungarian sediments was first published by E. KRIVÁN-HUTTER (1963). Later several LM data were published from different localities and ages. After the discovery of a great amount of Alginite in Transdanubia in Hungary multidisciplinary researches started on this sediment. The most important results are as follows: The oil shale (Alginite) from Hungary was first discussed by JÁMBOR and SOLTI (1975). Geology: JÁMBOR (1980), HETÉNYI (1985), JÁMBOR and SOLTI (1975, 1976), SOLTI (1981). Geology and Mineralogy: MEZŐSI (1976), Petrography: RAVASZ (1976). Ecology: E. NAGY (1976), HAJÓS (1976), JÁMBOR (1980). Geochemistry: ARATÓ and BELLA (1976), HETÉNYI and VARSÁNYI (1976), GRASSELLY, BERTALAN and SAJGÓ (1977), HETÉNYI, MAITZ and TÓTH (1977), HETÉNYI (1979, 1980, 1985, 1987-1988), HETÉNYI and PÁPAY (1986), HETÉNYI and SIROKMÁN (1978), plant macrofossils: KVAČEK, HABLY and SZAKMÁNY (1994), palynology by E. NAGY (1965, 1975a,b, 1992, 1993, 1997).

The aim of this paper is to compare the results of the different methods, and to establish, whether the resolution of the used SEM instrument and the method is suitable to demonstrate the larger biopolymer units, which may be modelled by the fullerenes. We must emphasize the important methodical differences between the TEM and SEM instruments used. It may be presumed that the data of the SEM instruments of a resolution power more or less identical with the TEM instrument or at least below 10 Å, and without metal covering will bring more exact data in this respect, but we hope that our data obtained with the instruments of our present day opportunities will be useful for the further investigations on this really interesting and important subject.

Previous experimental investigations

KEDVES (1986a) published the first TEM results of the partially degraded colonies of *Botryococcus braunii* KÜTZ. isolated from Hungarian oil shale. Globular macromolecular units were observed, which may be arranged into filaments or are in irregular position. Results of combined investigations (LM, TEM and thin layer chromatography) were published by KEDVES (1986b). By the way of comparative thin layer chromatography molecular remnants of chlorophyllids, chlorophylls, carotenoids, ?lutein and two unidentifiable components between chlorophyllids and violaxanthin were established.

KEDVES (1987) concerning the studies of the degradation of the sporoderm under natural and in vitro conditions pointed out, that there are similarities between fossil angiosperm exines and the walls of fossil algae, e.g. *Botryococcus* and *Pleurozonaria*. KADOURI, DERENNE, LARGEAU, et al. (1988), DERENNE, LARGEAU, CASADEVALL and BERKALOFF (1989) published TEM picture of treated recent colonies for isolating PRB.

DUBREUIL, DERENNE, LARGEAU, BERKALOFF and ROUSSEAU (1989) published TEM data from the Darwin Coorongite and saponified *Botryococcus braunii* B race colonies. Concentric lamellae were described. DERENNE, METZGER, LARGEAU, et al. (1991) established similar morphological variations on *Gloeocapsomorpha prisca* in Ordovician sediments and cultured *Botryococcus braunii* in consequence of the changes in salinity.

TEMPLIER, LARGEAU, CASADEWALL and BERKALOFF (1992) published SEM and TEM pictures from untreated, after lipid extraction, after basic hydrolysis from the A and B races of *Botryococcus* colonies (recent). Characteristic lamellar structures of the wall are illustrated in picture Bi, Fig. 5., after lipid extraction. KEDVES, ROJIK and VÉR (1992) concerning the biopolymer organization of the *Botryococcus* colonies from Hungarian Alginite pointed out the following; p. 21: "The experiments were made in the following ways: 1. Partial dissolution and degradation of the colonies with NaOH, 2-aminoethanol, $KMnO_4$ aq. dil. and with combination of the two latter mentioned chemicals. 2. Protoplast method (HELIX enzymatic destruction)".

KEDVES, TÓTH and FARKAS (1993) emphasized that the large globular units described first by KEDVES, ROJIK and VÉR (1991) can be equated with the recently discovered fullerenes.

KEDVES, TÓTH and VÉR (1993) presented first the radial fivefold rotation, and the term of the rotation areas was introduced, and alterations and secularities of the rotation areas, and the importance of the extra-areal secondary points of symmetries were published as a preliminary report.

VÉR (1994) used the LM method to investigate the acceptance of different stains of the *Botryococcus* colonies from Pula, and she emphasized, that this method is suitable to establish the degrees of maturity of the organic components of the colonies.

KEDVES, TÓTH and VÉR (1993, 1995) published in detail the radial fivefold rotation method on the partially degraded quasi-periodic biopolymer structures of the wall of *Botryococcus* colonies isolated from the Alginite of Pula. For the first time two kinds of radial rotations were elaborated. In this paper the rotation area was also published secular alterations in the size and the shape of the rotation areas were established.

KEDVES, TRIPATHI, VÉR, PÁRDUTZ and ROJIK (1998) carried out new symmetry operations on a partially degraded and fragmented colony of *Botryococcus braunii* KÜTZ. On this fragment the quasi-periodic and quasi-equivalent biopolymer structures were established. Our pentagon biopolymer units were chosen from the bordering zone of these two kinds of symmetries. Different relationships were established between the quasi-crystalloid and quasi-equivalent biopolymer system. After partial degradation with 2-aminoethanol and merkaptoethanol for 30, 60, 90 and 150 days the molecular system sensu strictu was dissolved.

Materials and Methods

The investigation material came from Pula from the collection of Prof. Dr. M. HETÉNYI.

The partial dissolution and degradation experiments were made in the Cell Biological and Evolutionary Micropaleontological Laboratory as follows:

3 mg dry *Botryococcus* colonies were used for each experiment, at 30 °C temperature.

AKP-99-1. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 24 h.

AKP-99-2. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 48 h.

AKP-99-3. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 72 h.

AKP-99-4. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 24 h, after washing + 10 ml $KMnO_4$ 1%, length of time 24 h.

AKP-99-5. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 48 h, after washing + 10 ml $KMnO_4$ 1%, length of time 24 h.

AKP-99-6. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 72 h, after washing + 10 ml $KMnO_4$ 1%, length of time 24 h.

AKP-99-7. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 24 h, after washing + 1 ml merkaptoethanol, length of time 24 h.

AKP-99-8. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 48 h, after washing + 1 ml merkaptoethanol, length of time 24 h.

AKP-99-9. - 3 mg *Botryococcus* colony + 1 ml 2-aminoethanol, length of time 72 h, after washing + 1 ml merkaptoethanol, length of time 24 h.

For LM investigations the colonies were mounted in glycerine-jelly, hydrated at 39.6%. For SEM investigations the dry organic material was covered with gold-palladium, the pictures were taken in the SEM Laboratory of the Department of Botany of the University of Szeged on a Hitachi S-2400 instrument, resolution about 40 Å. For TEM investigations the partially dissolved and degraded colonies were embedded in Araldite without fixation with OsO_4 aq. dil. The TEM studies were made in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences. For the ultrathin sections Porter Blum ultramicrotome with glass knives was used. The TEM photographs were taken on a Zeiss EM-902 (resolution 2-3 Å) and on a Tesla BS-540, resolution 6-7 Å.

Results

In Plate 5.1. non-experimental LM (fig. 1) and TEM (fig. 2) picture represents the basic morphology and ultrastructure of the *Botryococcus braunii* colonies isolated from the alginite of Pula. The light microscopical picture well illustrate the relatively long pedunculus and the cupules. The basic ultrastructure of the well preserved non-experimental wall is compact with tiny holes and sometimes differences in the electron affinity of the outer wall are illustrated (Plate 5.1., fig. 2). To the latter mentioned picture on the negative of a previously published (KEDVES, 1983) matter was used.

As a basic comprehensive material we publish again a TEM picture (Plate 5.2.) of the partially degraded and fragmented wall of the *Botryococcus* colonies, which was first published by KEDVES, ROJIK and VÉR (1991). Later it was pointed out that the large globular biopolymer units may be modelled with the fullerenes.

The diameter of the smaller globular units was measured recently, the results are as follows:

20	24	28	32	36	40	44	48	52	56	60	Å
21.4	20.0	12.9	11.4	7.0	8.6	3.6	5.7	2.9	3.6	2.9	%

The diameter of the large globular units is about 224 - 240 Å.

1. LM results

Experiments: AKP-99-1-3 (Plate 5.3., figs. 1-3)

After partial dissolution with 2-aminoethanol during 1-2-3 days no important alterations were established in the light microscopical morphology. The only thing is, that this method was not suitable to demonstrate the presence of the characteristic mucilage on the surface.

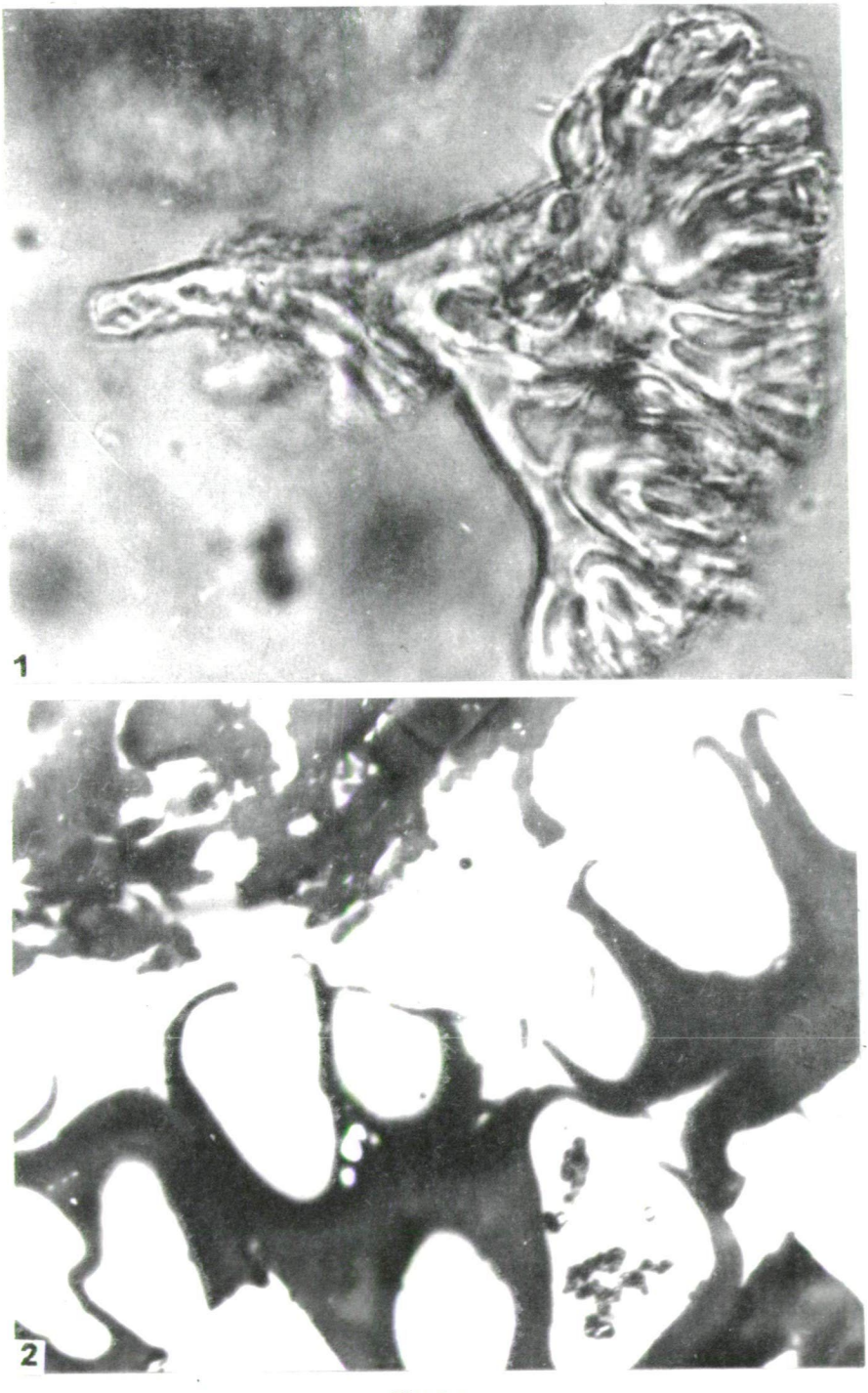

Plate 5.1.

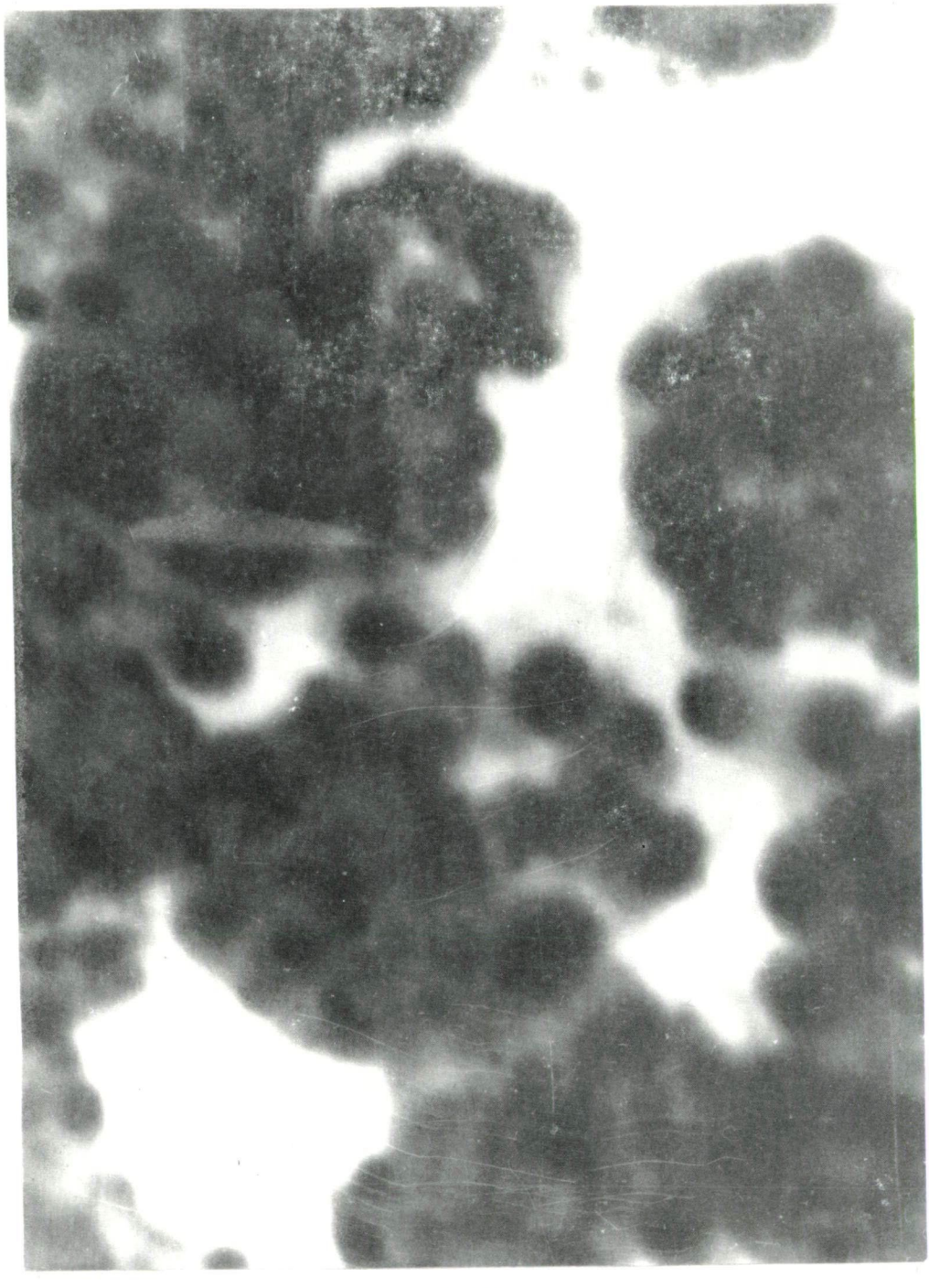

Plate 5.2.

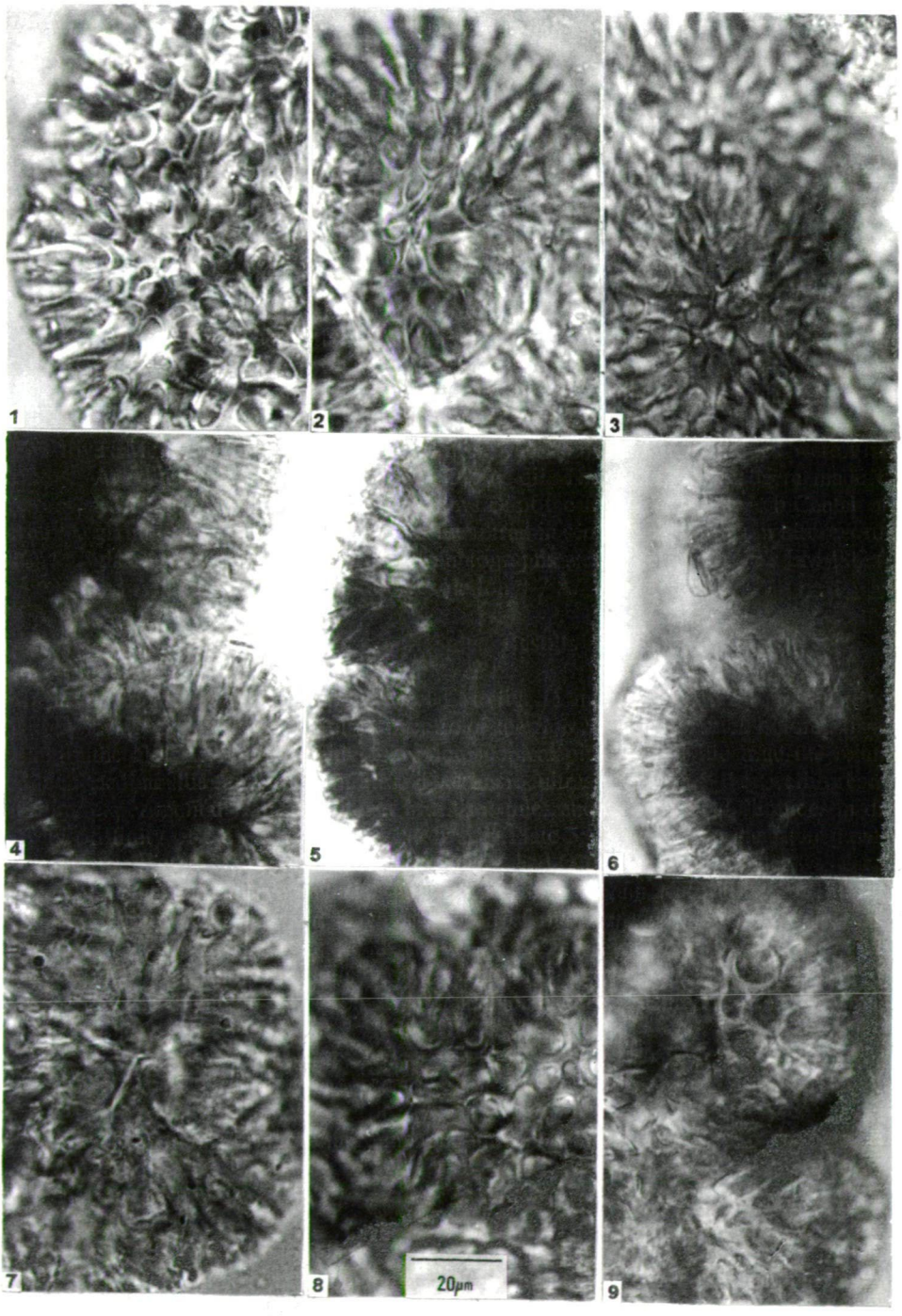

Plate 5.3.

Experiments: AKP-99-4-6 (Plate 5.3., figs. 4-6)

The oxidation after partial dissolution with 2-aminoethanol resulted in important alterations in the light microscopy of the colonies. The gradual degradation of the outer part of the colonies is well shown in pictures 4,5,6 in Plate 5.3. The inner part of the colonies is very dark, sometimes dark granules are present in the outside cupules of the colonies.

Experiments: AKP-99-7-9 (Plate 5.3., figs. 7-9)

The effect of the merkaptoethanol after 2-aminoethanol is well shown by the LM method too. A peculiar dissolution may be established, the walls of the cupules is not so characteristic in comparison with the experiment AKP-99-1 (Plate 5.3., fig. 1). Electron dense particles may be observed in the cupules in picture 7 of Plate 5.3.

2. EM results

Experiment: AKP-99-1 (Plate 5.4., figs. 1,2, plate 5.5., figs. 1,2)

SEM pictures (Plate 5.4., figs. 1,2) illustrate well the surface of the cupulae and the outer part of the wall. Remnants of the mucilage are well shown. Alterations in the ultrastructure of the wall were established with the transmission electron microscopical method (Plate 5.5., figs. 1,2). In the picture of low magnification (Plate 5.5., fig. 1) different kinds of electron dense particles were observed. These are granular, units separated or in linear or irregular arrangement (Plate 5.5., fig. 2). The general survey of this substance of the partially degraded wall is spongy.

Plate 5.1.

1,2. *Botryococcus braunii* KÜTZ. non experimental colonies.
1. LM picture of a colony. Illustrated are the relatively long pedunculus and the lateral view of the cupules. Slide: Pula-II-00-1, 1.200x.
2. TEM picture of the colony. Negative No: 3009, 2.500x.

Plate 5.2.

Biopolymer structure of the partially degraded and fragmented wall of *Botryococcus braunii* KÜTZ. Experiment No: 925, negative No: 0596, 250.000x.

Plate 5.3.

1-9. *Botryococcus braunii* KÜTZ. LM pictures of the partially degraded colonies.
1. Experiment No: AKP-99-1.
2. Experiment No: AKP-99-2.
3. Experiment No: AKP-99-3.
4. Experiment No: AKP-99-4.
5. Experiment No: AKP-99-5.
6. Experiment No: AKP-99-6.
7. Experiment No: AKP-99-7.
8. Experiment No: AKP-99-8.
9. Experiment No: AKP-99-9.

Experiment: AKP-99-2 (Plate 5.4., figs. 3-5, plate 5.5., figs. 3,4).

The magnified SEM pictures (Plate 5.4., figs. 4,5) illustrate globular units on the surface. The diameter of these units is as follows:

20	30	40	50	60	70	80	90	100	Å
28.6	22.1	7.8	14.3	7.8	6.5	9.0	2.6	1.3	%

It seems that based on the diameter the superficial globular units may be classed into two groups: 1. 20-30 Å, 2. 50-100 Å. This difference is well illustrated in picture 5, Plate 5.4. In the TEM pictures within the cupules electron dense particles were observed which may be remnants of the kerogens, e.g. fig. 3, in Plate 5.5. Not characteristic lamellar structure was also observed. On the highly magnified pictures (Plate 5.5., fig. 4) electron dense granular structures were also observed in the less electron dense substance.

Experiment: AKP-99-3 (Plate 5.4., figs. 6-8, plate 5.5., figs. 5,6)

In the highly magnified SEM pictures lamellar structures in the wall of the cupules (Plate 5.4., fig. 7) and destruction of the superficial layers were observed (Plate 5.4., fig. 8). Characteristic superficial globular units were not observed in contrast to the previous experiment. Based on the transmission electronmicroscopical results the degradation of the wall is advanced (Plate 5.5., figs. 5,6). There are globular electron dense particles in the wall of the cups (Plate 5.5., fig. 6).

Experiment: AKP-99-4 (Plate 5.6., figs. 1-3, plate 5.7., figs. 1,2)

The surface of the cups is more or less smooth, with remnants of the mucilage or other organic debris (Plate 5.6., fig. 3). Occasionally not so characteristic lamellar wall structure was observed (Plate 5.6., fig. 2). The lamellar structure of the wall is well illustrated on the TEM pictures (Plate 5.7., fig. 1). There are differences in the electron density of the lamellae. The different organization levels of the molecular structure are well shown in the highly magnified pictures (Plate 5.7., fig. 2). The per cents of the diameter of the globular units are as follows:

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Å
1.4	19.8	18.0	27.0	9.5	6.7	4.0	-	-	-	2.7	4.0	2.7	-	-	-	-	1.4	1.4	-	-	1.4	%

This experiments was the best for molecular symmetry operations, which will be made in the future.

Experiment: AKP-99-5 (Plate 5.6., figs. 4-6, plate 5.7., figs. 3,4)

Based on the SEM data the lamellar structure of the wall is not characteristic. (Plate 5.6., fig. 5). In the highly magnified SEM pictures the larger globular molecular units are well presented.

The percentages of the different diameter of the globular units are the following:

20	30	40	50	60	Å
16.5	42.2	29.3	11.2	0.8	%

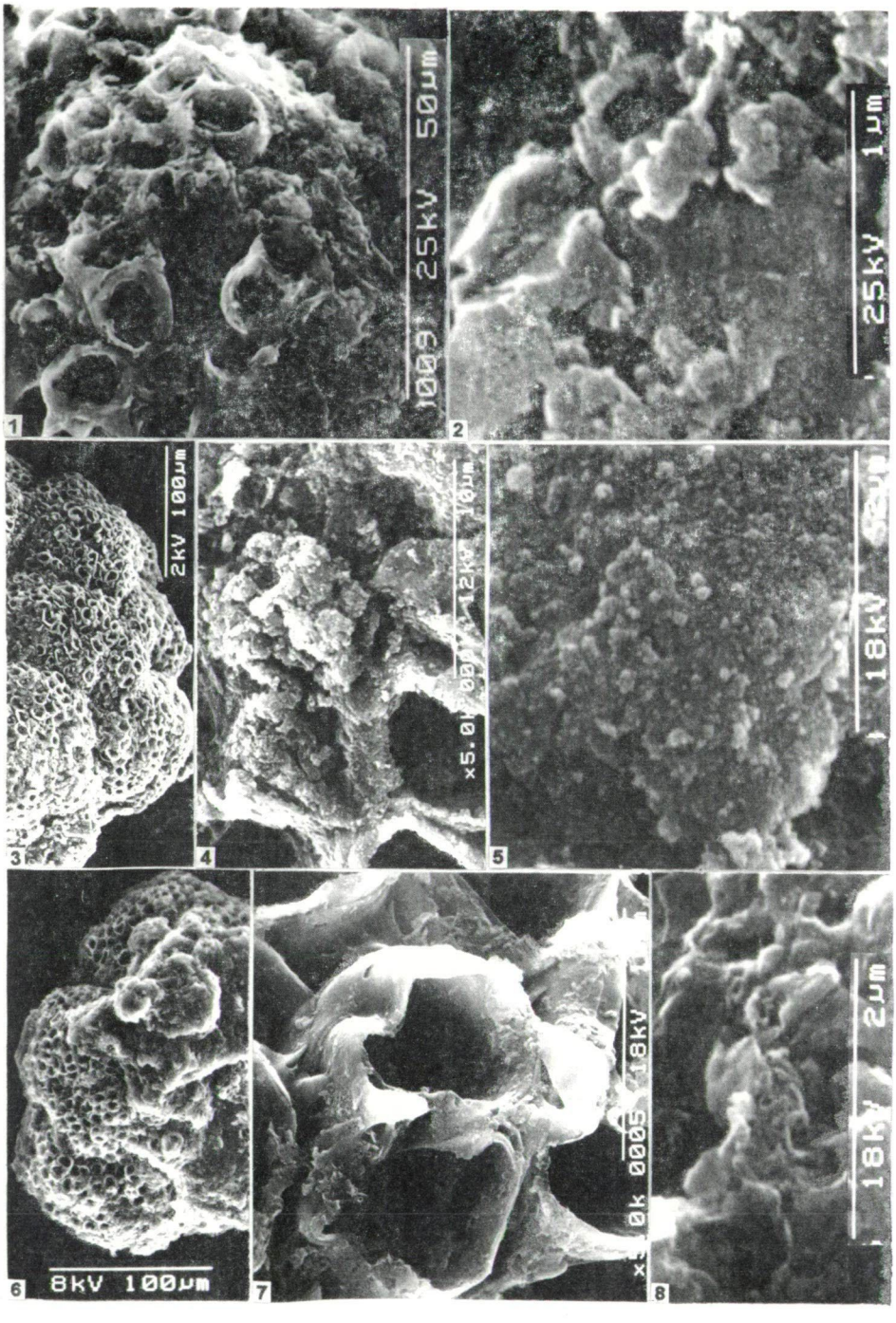
1
1009 25kV 50µm
2
25kV 1µm
3
2kV 100µm
4
5
18kV
6
8kV 100µm
7
8
18kV 2µm

Plate 5.4.

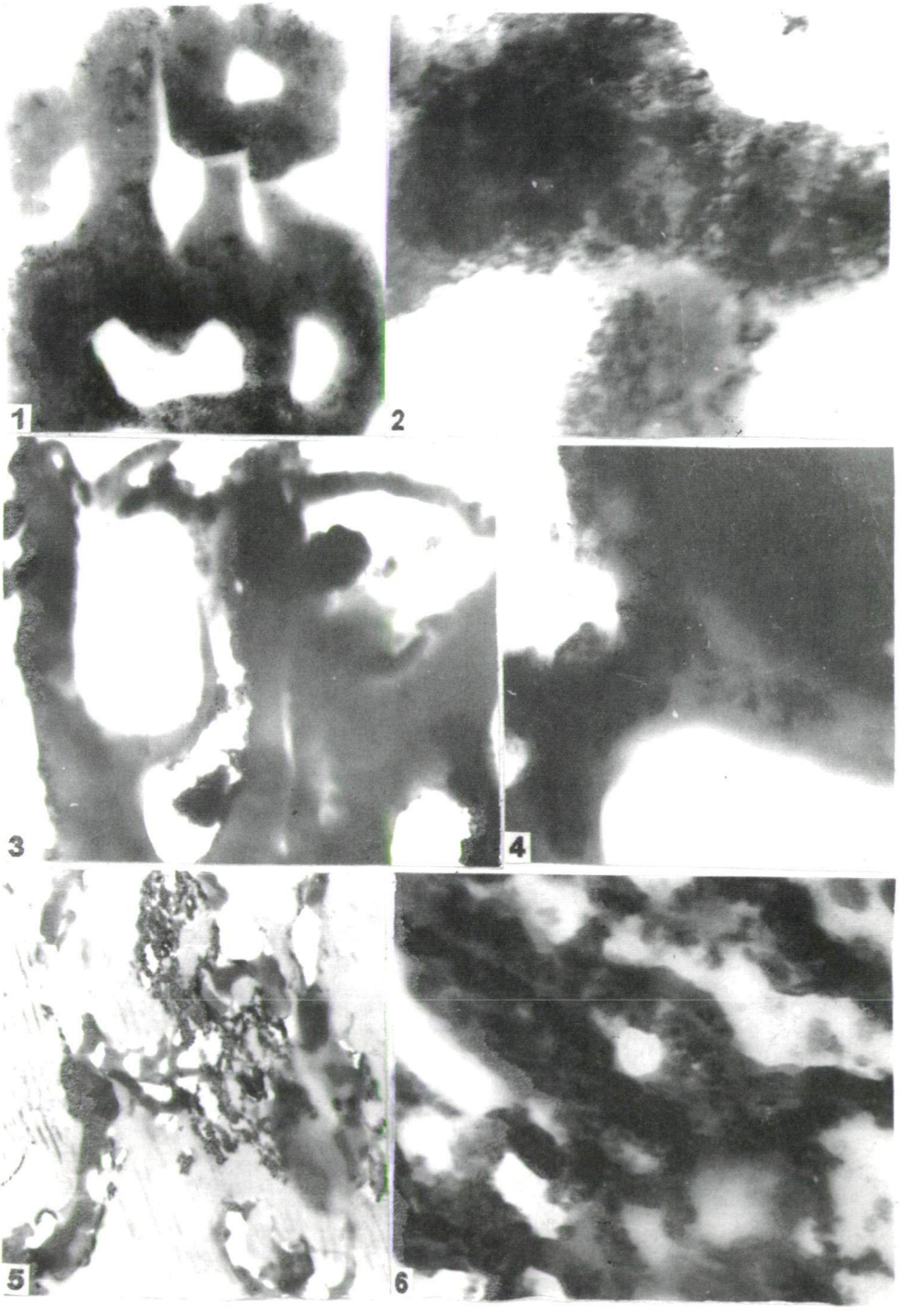

Plate 5.5.

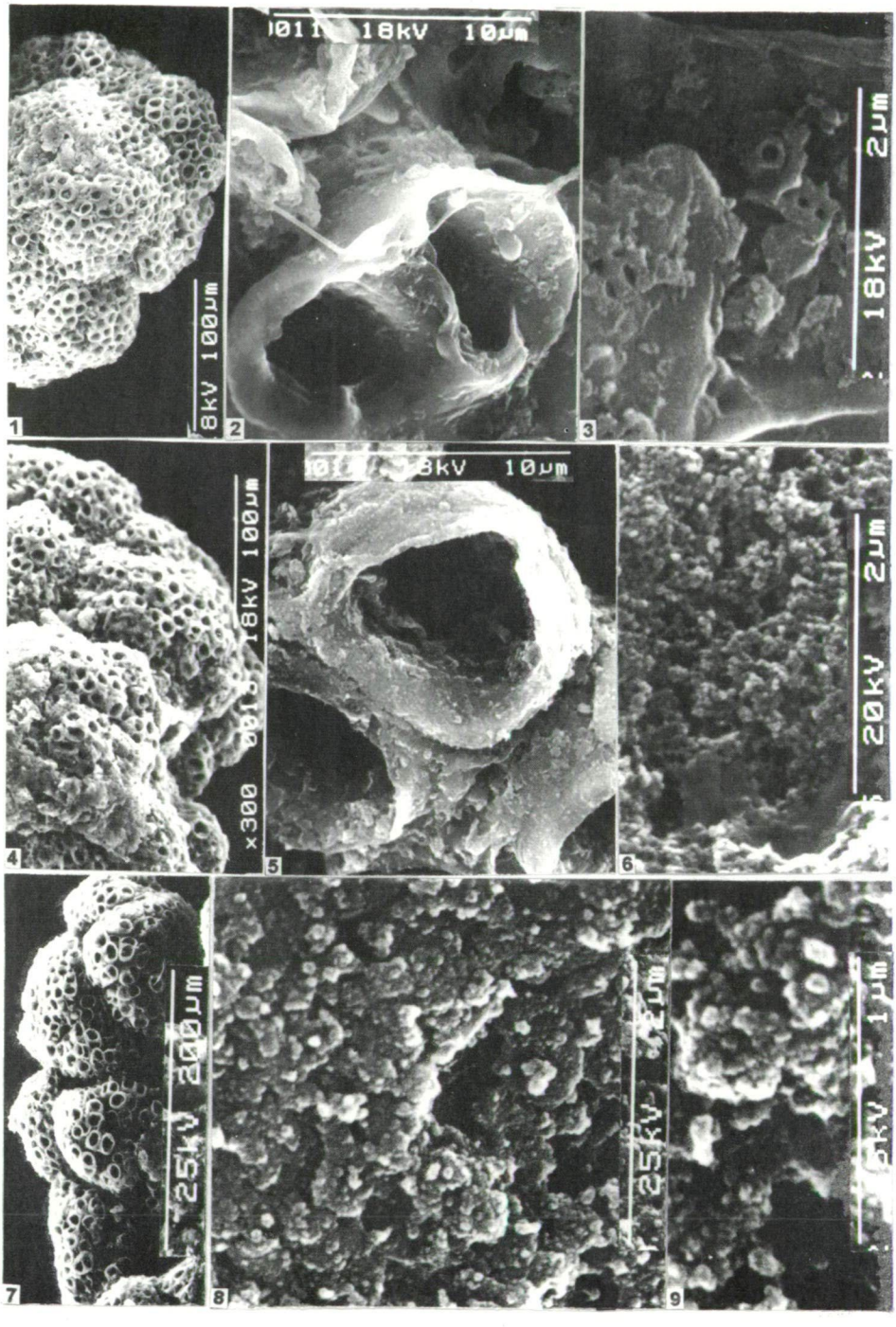
1
8kV 100µm
2
18kV 10µm
3
18kV 2µm
4
×300
18kV 100µm
5
8kV 10µm
6
20kV 2µm
7
25kV 200µm
8
25kV
9
1µm

Plate 5.6.

Plate 5.4.

1-8. *Botryococcus braunii* KÜTZ. SEM. pictures of the partially degraded colonies.
1,2. Experiment No: AKP-99-1.
3-5. Experiment No: AKP-99-2.
6-8. Experiment No: AKP-99-3.

Plate 5.5.

1-6. *Botryococcus braunii* KÜTZ. TEM pictures of the partially degraded colonies.
1,2. Experiment No: AKP-99-1. 1. Negative No: 7085, 15.000x, 2. Negative No: 7806, 50.000x.
3,4. Experiment No: AKP-99-2. 3. Negative No: 7773, 5.000x, 4. Negative No: 7774, 15.000x.
5,6. Experiment No: AKP-99-3. 5. Negative No: 7778, 5.000x, 6. Negative No: 7776, 50.000x.

Plate 5.6.

1-9. *Botryococcus braunii* KÜTZ. SEM pictures of the partially degraded colonies.
1-3. Experiment No: AKP-99-4.
4-6. Experiment No: AKP-99-5.
7-9. Experiment No: AKP-99-6.

The TEM pictures illustrate well the characteristic lamellar ultrastructure of the wall (Plate 5.7., fig. 3). The kerogen content of the cup was also observed. There are differences in the electron density in this organic content. Worth of mentioning is, the degradation of the molecular system (Plate, 5.7., fig. 4). The diameters of the more or less well preserved globular units are as follows:

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Å
11.5	16.3	21.2	24.0	2.9	4.8	5.8	3.8	3.8	1.0	-	-	-	1.0	1.9	-	1.0	1.0	%

Experiment: AKP-99-6 (Plate 5.6., figs. 7-9, plate 5.7., figs. 5,6)

Autospores are illustrated in the general survey SEM pictures (Plate 5.6., fig. 7). The globular biopolymer structures of the surface are well shown in the highly magnified SEM pictures. The percentages in the size are as follows:

20	30	40	50	60	70	80	90	100	110	120	Å
13.3	26.2	18.9	15.1	12.0	6.9	4.4	1.3	1.3	-	0.6	%

The general survey TEM picture illustrates well the lamellar system of the cups. The electron density of the different lamelles are characteristic. In some part of the ultrathin sections globular biopolymer units of different size were measured. The percentages of the diameter of the smaller globular units are as follows:

20	30	40	50	60	70	Å
0.5	14.1	36.7	35.0	9.2	4.5	%

Based on the measurement of few large globular units, the diameter is about 210-250 Å.

Experiment: AKP-99-7 (Plate 5.8., figs. 1-3, plate 5.9., figs. 1,2)

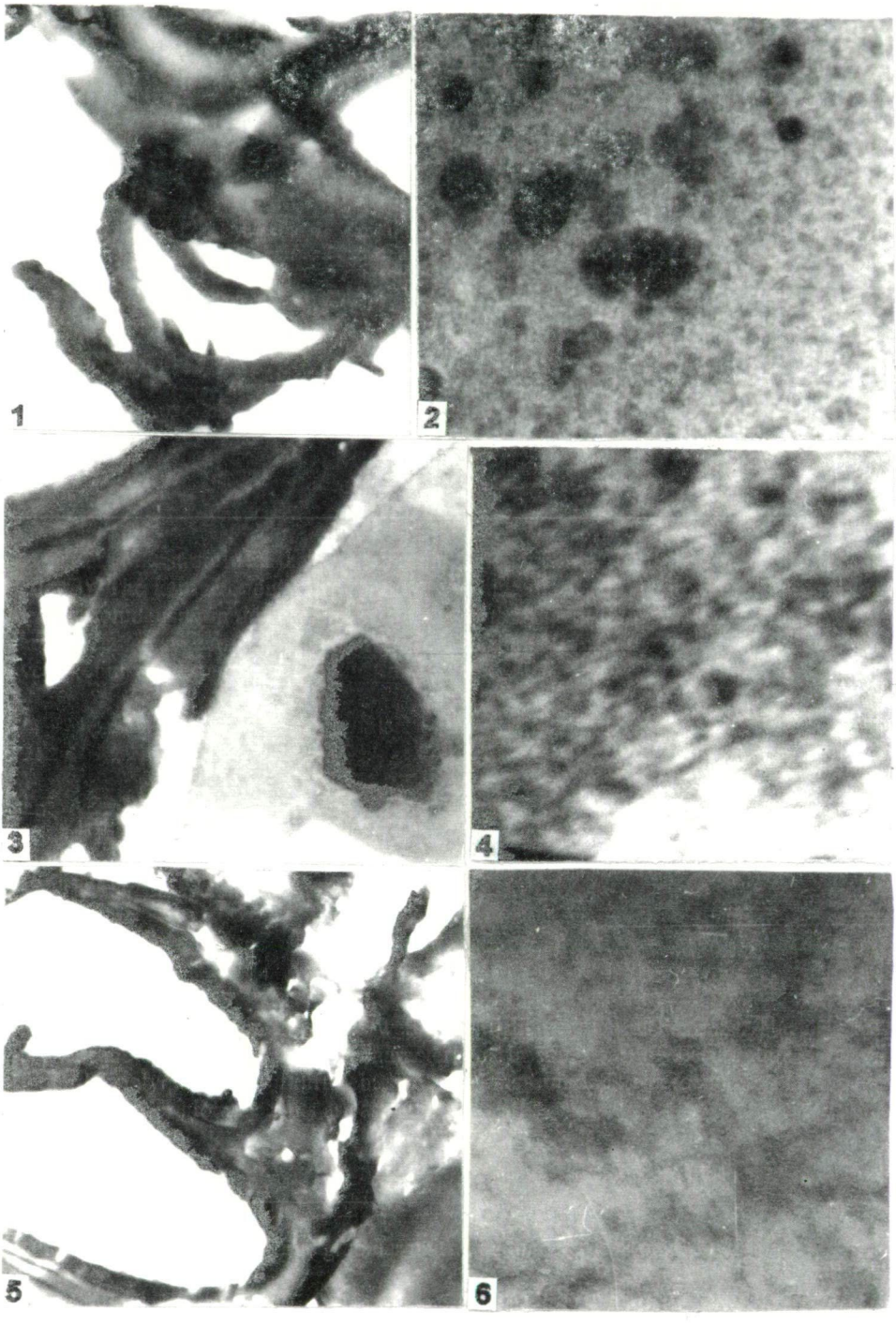

Plate 5.7.

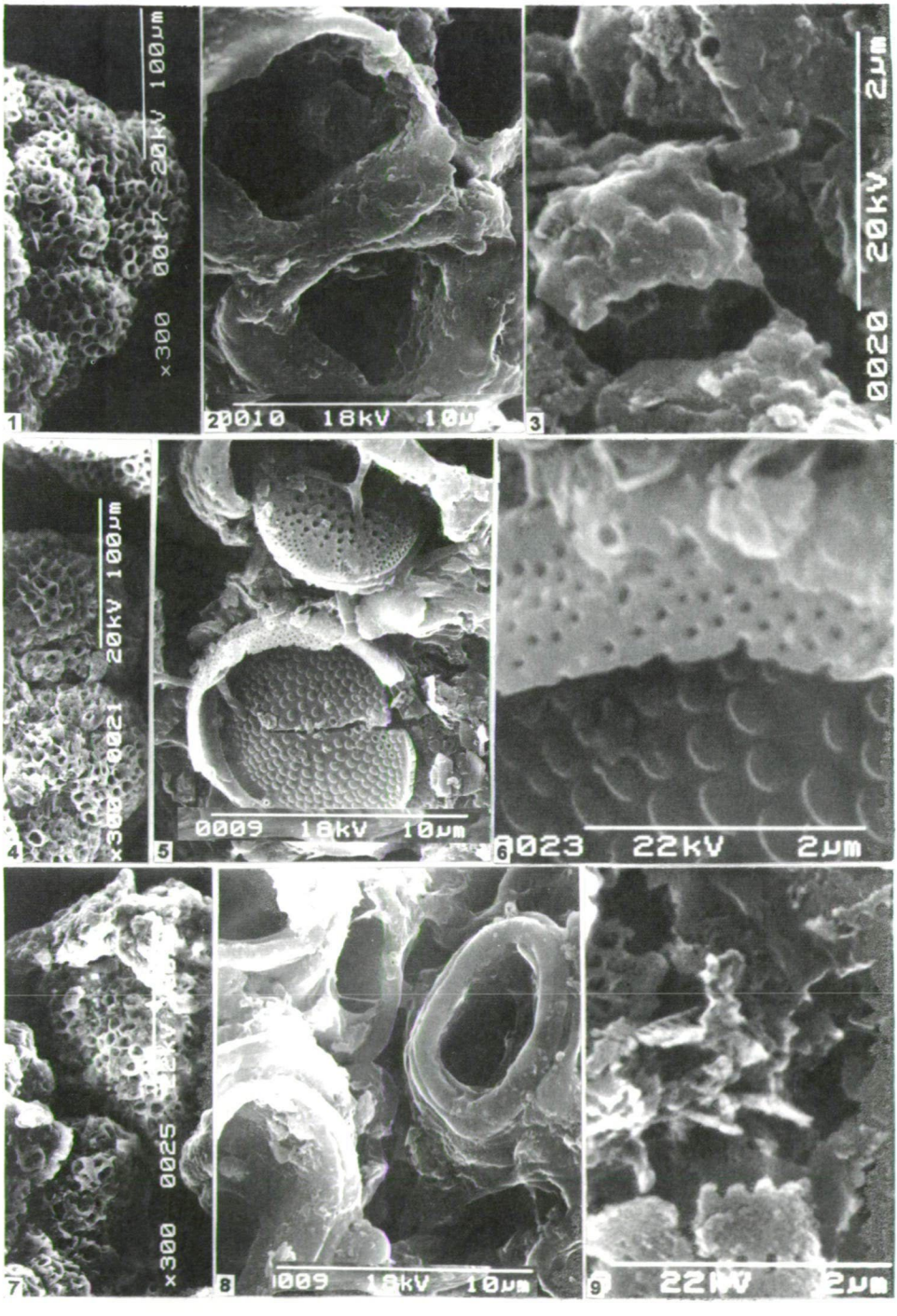
1
×300 0017 20kV 100µm
2 0010 18kV 10µm
3
0020 20kV 2µm
4
20kV 100µm
5
0009 18kV 10µm
6
0023 22kV 2µm
7
×300 0025
8
1009 18kV 10µm
9
22kV 2µm

Plate 5.8.

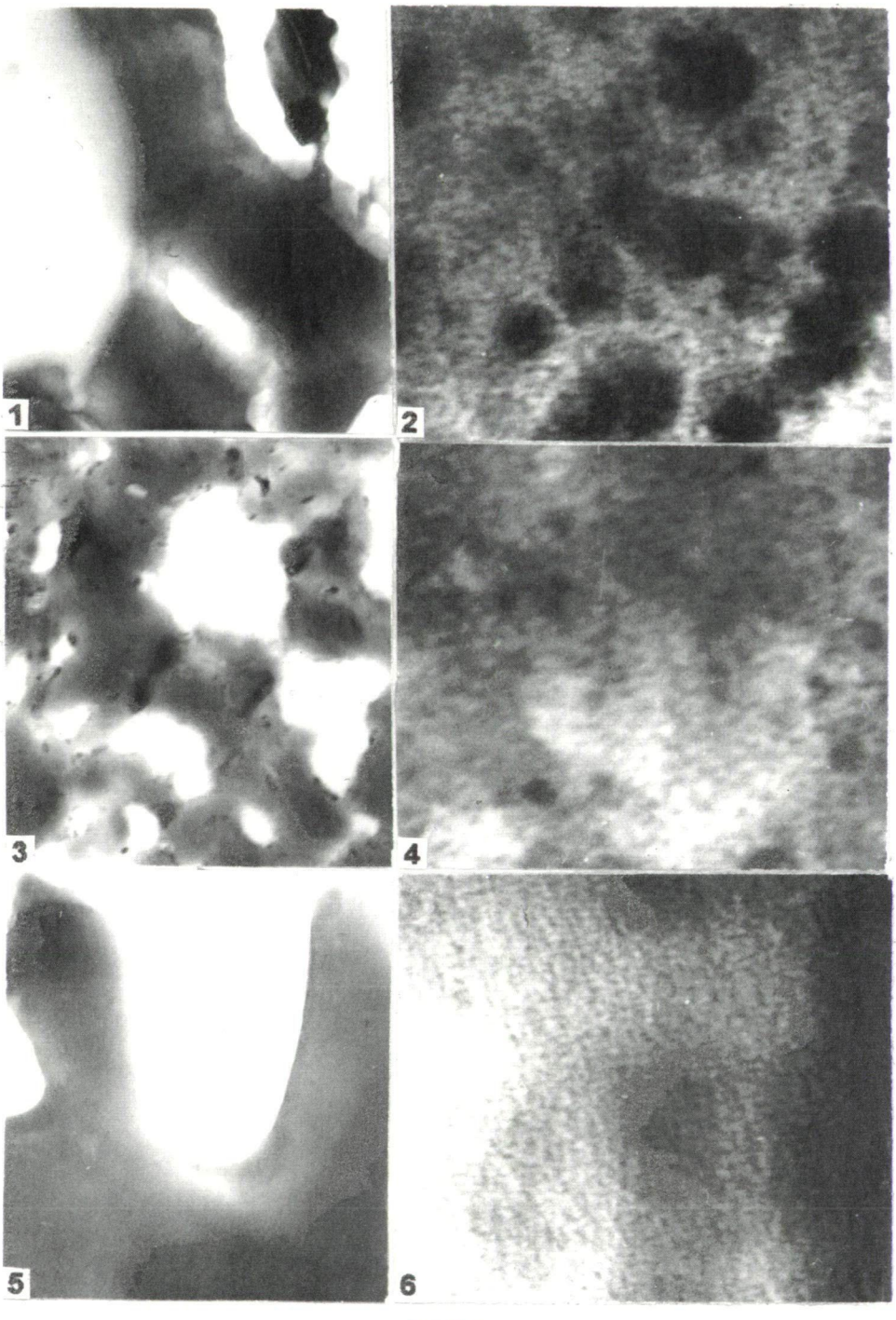

Plate 5.9.

Autospores are illustrated in the general survey SEM pictures (Plate 5.8., fig. 1). The highly magnified superficial pictures illustrate more or less smooth surface with some organic debris (Plate 5.8., figs. 2,3). In the low magnified TEM picture more or less homogeneous wall was observed (Plate, 5.9., fig. 1). Highly magnified TEM picture revealed the different kinds of molecular systems. The size of the globular units is as follows:

30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	Å
10.0	6.0	12.0	10.0	2.0	10.0	10.0	4.0	12.0	4.0	12.0	-	2.0	-	-	-	-	4.0	-	2.0	%

Experiment: AKP-99-8 (Plate 5.8., figs. 4-6, plate 5.9., figs. 3,4)

Autospores are well seen in the general survey SEM picture (Plate 5,8., fig. 4). In the highly magnified pictures perforations in the inner, third cup were observed. The diameter of these perforations is as follows:

20	30	40	50	60	70	Å
9.7	20.2	41.1	21.0	6.4	1.6	%

The superficial ornamentation of the cell cup or the cup content are illustrated in picture 5 and 6, plate 5.8. The diameter of the ornamental elements is 150 - 290 Å, average 216 Å.

There are perforations on the ornamental elements of 10 - 20 Å in size.

The general survey TEM picture illustrates a more or less homogeneous wall with small electron dense particles. There are globular or elongated electron dense small particles (Plate 5.9., fig. 3). In the highly magnified picture globular biopolymer units are well presented, the degradation of the larger globular units is well shown (Plate 5.9., fig. 4).

20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	Å
7	34	22	11	10	3	4	3	3	-	-	1	-	-	-	1	-	-	-	-	1	%

Plate 5.7.

1-6. *Botryococcus braunii* KÜTZ. TEM pictures of the partially degraded colonies.
1,2. Experiment No: AKP-99-4. 1. Negative No: 7769, 15.000x. 2. Negative No: 8623, 500.000x.
3,4. Experiment No: AKP-99-5. 3. Negative No: 7767, 15.000x. 4. Negative No: 8620, 1.000.000x.
5,6. Experiment No: AKP-99-6. 5. Negative No: 7784, 5,000x. 6. Negative No: 7751, 100.000x.

Plate 5.8.

1-9. *Botryococcus braunii* KÜTZ. SEM pictures of the partially degraded colonies.
1-3. Experiment No: AKP-99-7.
4-6. Experiment No: AKP-99-8.
7-9. Experiment No: AKP-99-9.

Plate 5.9.

1-6. *Botryococcus braunii* KÜTZ. TEM pictures of the partially degraded colonies.
1,2. Experiment No: AKP-99-7. 1. Negative No: 7763, 15.000x. 2. Negative No: 8616, 1,000.000x.
3,4. Experiment No: AKP-99-8. 3. Negative No: 7759, 15.000x. 4. Negative No: 8612, 1,000.000x.
5,6. Experiment No: AKP-99-9. 5. Negative No: 7754, 50.000x. 6. Negative No: 8608, 1,000.000x.

Experiment: AKP-99-9 (Plate 5.8., figs. 7-9, plate 5.9., figs. 5,6)

Autospores are illustrated in the general survey SEM picture (Plate 5.8., fig. 7). Not so characteristic lamellar structure of the cups and different kinds of organic remnants on the surface are illustrated in the highly magnified SEM pictures (Plate 5.8., figs. 8,9). The degradation of the biopolymer structure of the wall may be emphasized on the basis of the highly magnified TEM picture (Plate 5.9., fig. 6).

Discussion and Conclusions

1. In the first place it is necessary to emphasize the heterogeneous character of our experimental material. Differences are in the ontogenetic stages of the colonies, in this way the molecular system is different, consequently the alterations during the sedimentation processes are also different. Of course this has an effect on the results of the experiments. This problem was emphasized by GUY-OHLSON and LINDQVIST (1990), too, and illustrated with SEM pictures from *Botryococcus* colonies of different stages of development and state of preservation. The new results concerning the molecular system are also worth of mentioning at this question, namely the biopolymer of these *Algae* is named PRB (Polymère Resistant de *Botryococcus*) LARGEAU, CASADEVALL, KADOURI and METZGER (1984), DERENNE, LARGEAU and CASADEVALL (1991), algaenan; cf. TEGELAAR, DE LEEUW, DERENNE and LARGEAU (1989), LARGEAU, DERENNE, CASADEVALL et al. (1990), BRENNER, (1998), DE LEEUW, VAN BERGEN et al. (1991) botryococcene highly unsatured isoprenoid hydrocarbons (DUBREUIL, DERENNE, LARGEAU et al. (1989), respectively botryococcane (fossil biopolymer) to distinguish from the other sporopollenin type biopolymer systems (e.g.: DERENNE, LARGEAU, CASADEVALL and CONNAN (1988 a,b), DUBREUIL, DERENNE, LARGEAU et al. (1989), BRENNER (1998), etc.).

2. Our LM results on the non-coloured colonies may be summarized as follows: The 2-aminoethanol (experiments AKP-99-1-3) "cleaned" the surface of the cups. The oxidation with $KMnO_4$ (experiments AKP-99-4-6) revealed dark content within the cups. Finally the experiments AKP-99-7-9, by 2-aminoethanol and merkaptoethanol resulted in not so characteristic degradations on the cups.

3. As regards the EM results in the first place it is necessary to emphasize that there are very important methodical differences and problems. The TEM pictures were taken with instruments of 6-7 Å, respectively 2-3 Å, and the colonies were embedded without OsO_4 aq. dil. postfixation. The resolution of the used SEM instrument is about 40 Å, and the material of investigations was covered with gold-palladium. We need to point out these basic methodical problems, together with the heterogeneous character of our experimental material, however we try to start the evaluation of the data obtained by different methods. We know well that the present day conclusions will be modified or changed in the future based on the new SEM data of instrument with more much better resolution (below 10 Å) and without metal covering.

3.1. SEM results:

3.1.1. The degradation of the mucilage started with the dissolution with 2-aminoethanol during 24 h.

3.1.2. Superficial lamellae respectively and different cups respectively were observed at different kinds of experiments (Plate 5.4., fig. 7, plate 5.6., fig. 2, plate 5.8., fig. 8). It seems that in this case the maturity or the diagenesis of the colonies is an important factor in this result. DERENNE, LARGEAU, HETÉNYI et al. (1997) based on the SEM in-

vestigations of the untreated colonies of *Botryococcus braunii* from Pula distinguished thick multilayered, and thinner and less layered outer walls.

3.1.3. Larger biopolymer globular systems were observed in the first place after the combined degradation with 2-aminoethanol and $KMnO_4$ (Plate 5.6., figs. 6,8,9) but occasionally with 2-aminoethanol only during 48 hours (Plate 5.4., fig. 5). Perforations of the cup and ornamentation of the surface of the probably kerogen content of the cell cup was observed till this time at one experiment.

3.2. TEM results

Characteristic lamellar ultrastructure of different electron density was observed after experiments with 2-aminoethanol and $KMnO_4$ (Plate 5.7., figs. 1,3,5). The organization of the cups (first, second and third) may be well recognized (cf. BATTEN and GRENFELL, 1996). Different kinds of electron dense particles were observed within the wall of the cups in the first place at the experiments AKP-99-1-3, and 8.

4. Evaluation of the different organization levels of the globular biopolymer structures investigated by different methods.

4.1. The largest globular structures of diameter more than 130 Å.

4.1.1. TEM data of partially degraded and fragmented colonies (KEDVES, ROJIK and VÉR, 1991): 224-240 Å.

4.1.2. TEM data of the ultrathin sections of experiment AKP-99-6: 210-250 Å.

4.1.3. TEM data of the ultrathin sections of experiment AKP-99-7: 150-220 Å.

4.1.4. TEM data of the ultrathin sections of experiment AKP-99-8: 130-220 Å.

4.1.5. SEM data of experiment AKP-99-8: 150-290 Å.

4.1.6. For comparison we use the SEM data of the protectum of *Caesalpinia japonica* published by TAKAHASHI (1993, fig. 7, p. 196). The diameter of the globular units is as follows:

142.9	190.5	238.1	285.7	333.3	380.9	Å
14.1	38.8	24.8	14.9	4.1	3.3	%

These sporopollenin units are more or less in the category of our above discussed botryococcane biopolymer unit.

4.2. Globular structures of 20-130 Å diameter.

4.2.1. TEM data of the partially degraded and fragmented colonies (KEDVES, ROJIK and VÉR, 1991): 20-60 Å. These units are building elements of the above mentioned larger globular units.

4.2.2. TEM data of the ultrathin sections of experiment AKP-99-7: 30-130 Å.

4.2.3. SEM data of experiment AKP-99-2: 20-100 Å.

4.2.4. SEM data of experiment AKP-99-5: 20-60 Å.

4.2.5. SEM data of experiment AKP-99-6: 20-120 Å.

4.3. Globular structure of 2-20 Å.

4.3.1. TEM data of the ultrathin sections of experiment AKP-99-4: 2-23 Å.

4.3.2. TEM data of the ultrathin sections of experiment AKP-99-5: 2-19 Å.

The investigation of the further experiments is in progress. Trends in the alterations of the biopolymer structures may be established based on the following data. New symmetry operations are necessary to better understand this complicated biopolymer system.

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References

ARATÓ, K. and BELLA, M. (1976): Results of technological and chemical analyses of the oil shale of Pula and Gérce (Transdanubia, Hungary). - Annual Report of the Hungarian Geol. Inst. of 1974, 287-300.

BATTEN, D.J. and GRENFELL, H.R. (1996): 7 Green and Blue-Green Algae, Chapter 7D. *Botryococcus*. In: Palynology: principles and applications eds.: JANSONIUS, J. and MCGREGOR, D.C. - American Association of Stratigraphic Palynologists Foundation *1*, 205-214.

BERKALOFF, C., CASADEVALL, E.,LARGEAU,C., METZGER, P., PERACCA, S. and VIRLET, J. (1983): The resistant polymer of the walls of the hydrocarbon-rich alga *Botryococcus braunii*. - Phytochemistry *22*, 389-397.

BLACKBURN, K.B. and TEMPERLEY, B.N. (1936): *Botryococcus* and the algal coals. - Royal Soc. Edinburgh Trans. *58*, 841-868.

BRENNER, W.W.(1998): Grundlagen und Anwendungsmöglichkeiten der Mikro-absorptionsphotometrie für organische-wandige Mikrofossilien. - Geomar Report 76, 1-141.

BURNS, D.A. (1982): A transmission electron microscope comparison of modern *Botryococcus braunii* with some microfossils previously referred to that species. - Revista Española de Micropaleontologia 14, 166-185.

CHADEFAUD, M. et EMBERGER, L. (1960): Traité de Botanique. Systématique. Tome I: Les végétaux non vasculaires. Cryptogamie. - Masson et Cie Ed., Paris.

COLLINSON, M.E., VAN BERGEN, P.F., SCOTT, A.C. and DE LEEUW, J.W. (1994): The oil-generating potential of plants from coal and coal-bearing strata through time: a review with new evidence from Carboniferous plants. In: Coal and Coal-bearing strata as Oil-prone Source-Rocks eds.: SCOTT, A.C. and FLEET, A.J. - Geol. Soc. Spec. Publication *77*, 31-70.

COMBAZ, A. (1980): Les kerogènes vus au microscope. In: Kerogen insoluble organic matter from sedimentary rocks (ed.: B. DURAND), Edition Technip, Paris, 56-112.

COOKSON, I.C. (1953): Records of the occurrences of *Botryococcus braunii, Pediastrum* and *Hystrichosphaeridae* in Cainozoic deposits of Australia. - Mem. Nat. Mus. Vict. *18*, 107-123.

DE LEEUW, J.W., VAN BERGEN, P.F., VAN AARSSEN, B.G.K., GATELLIER, J.-P. L.A., SINNINGHE DAMSTÉ, J.S. and COLLINSON, M. E. (1991): Resistant biomacromolecules as major contributors to kerogen. - Phil. Trans. R. Soc. Lond. B *333*, 329-337.

DERENNE, S., LARGEAU, C., BERKALOFF, C., ROUSSEAU, B., WILHELM, C. and HATCHER, P.G. (1992): Non-hydrolysable macromolecular constituents from outer walls of *Chlorella fusca* and *Nannochlorum eucaryotum*. - Phytochemistry *31*, 1923-1929.

DERENNE, S., LARGEAU, C. and CASADEVALL, E. (1991): Occurrence of tightly bound isoprenoid acids in an algal, resistant biomacromolecule: possible geochemical implications. - Org. Geochem. *17*, 597-602.

DERENNE, S., LARGEAU, C., CASADEVALL, E. and BERKALOFF, C. (1989): Occurrence of a resistant biopolymer in the L race of *Botryococcus braunii*. - Phytochemistry *28*, 1137-1142.

DERENNE, S., LARGEAU, C., CASADEVALL, E., BERKALOFF, C. and ROUSSEAU, B. (1991): Chemical evidence of kerogen formation in source rocks and oil shales via selective preservation of thin resistant outer wall of microalgae: Origin of ultralaminae. - Geochimica et Cosmochimica *55*, 1041-1050.

DERENNE, S., LARGEAU, C., CASADEVALL, E. and CONNAN, J. (1988a): Comparison of torbanites of various origins and evolutionary stages. Bacterial contribution to their formation. Cause of the lack of botryococcanne in bitumens. - Org. Geochem. *12*, 43-59.

DERENNE, S., LARGEAU, C., CASADEVALL, E. and CONNAN, J. (1988b): Mechanism of formation and chemical structure of Coorongite II. Structure and origin of the labile fraction. Fate of botryococcenes during early diagenesis. - Org. Geochem. *13*, 965-971.

DERENNE, S., LARGEAU, C., CASADEVALL, E., TEGELAAR, E. and DE LEEUW, J.W. (1988): Relationships between algal coals, and resistant cell wall biopolymers of extant algae as revealed by Py-GC-MS. - Fuel Processing Technology *20*, 93-101.

DERENNE, S., LARGEAU, C., HETÉNYI, M., BRUKNER-WEIN, A., CONNAN, J. and LUGARDON, B. (1997): Chemical structure of the organic matter in a Pliocene maar-type shale: Implicated *Botryococcus* race strains and formation pathways. - Geochim. Cosmochim. Acta *61*, 1879-1889.

DERENNE, S., METZGER, P., LARGEAU, C., VAN BERGEN, P.F., GATELLIER, J.P., SINNINGHE DAMSTÉ, J.S., DE LEEUW, J.W. and BERKALOFF, C. (1991): Similar morphological and chemical variations of *Gloeocapsomorpha prisca* in Ordovician sediments and cultured *Botryococcus braunii* as a response to changes in salinity. - Org. Geochem. *19*, 299-313.

DUBREUIL, C., DERENNE, S., LARGEAU, C., BERKALOFF, C. and ROUSSEAU, B. (1989): Mechanism of formation and chemical structure of Coorongite-I. Role of the resistant biopolymer and of the hydrocarbons of *Botryococcus braunii*. Ultrastructure of Coorongite and its relationship with Torbanite. - Org. Geochem. *14*, 543-553.

DURAND, B. (1980): Kerogen insoluble organic matter from sedimentary rocks. – Editions Technip., Paris.

ERLSTRÖM, M. and GUY-OHLSON, D. (1994): Campanian depositional settings in the Vomb Trough, Scania, Sweden - GFF *116*, 193-202.

ERLSTRÖM, M., GUY-OHLSON, D. and SIVHED, U. (1994): Palaeoecology and sedimentary environments of the Jurassic-Cretaceous transition beds in Sweden. - Geobios *17*, 671-678.

FRÉMY P. et DANGEARD, L. (1938): Observations sur le *Botryococcus braunii* KÜTZING actuel et fossile. - Ann. Paléont. *27*, 115-136.

FRITSCH, F.E. (1935): The structure and reproduction of the *Algae*. Vol. 1. - Cambridge Univ. Press, Cambridge.

GAXIOLA, J.R. and SANTILLAN, M.A. (1986): The color of glycerinated alcohol of the palynologic residue indicates the generation, migration and accumulation conditions of liquid hydrocarbons in an oil basin. - Instituto Mexicano del Petroleo, México D.F.

GLIKSON, M., CHAPPELL, B.W. and WEBBERT, E. (1987): Trace element associations in raw and spent oil shales from the Rundle oil shale complex in Queensland. Environmental implications. - Fuel *66*, 400-403.

GLIKSON, M., LINDSAY, K. and SAXBY, J. (1989): *Botryococcus* - A planktonic green alga, the source of petroleum throught the ages: Transmission electron microscopical studies of oil shales and petroleum source rocks. - Org. Geochem. *14*, 595-608.

GRASSELLY, GY., BERTALAN, M. and SAJGÓ, CS. (1977): Contributions to the knowledge of the Hungarian oil shale kerogen II. Result of preliminary DTA and IR-investigations on the kerogen of the oil shale occurrence at Pula. - Acta Miner.-Petr. *23*, 165-175.

GRAY, J. (1960): Fossil *Chlorophycean* Algae from the Miocene of Oregon. - J. Paleont. *34*, 453-463.

GUY-OHLSON, D. (1986): Jurassic Palynology of the Vilhelmsfält Bore No. 1, Scania, Sweden Toarcian - Aalenian. - Section of Palaeobotany, Swedish Museum of Natural History, Stockholm.

GUY-OHLSON, D. (1990): Pliesbachian palynology of the Karindal bore no. 1, north-west Scania, Sweden. - Rev. Palaeobot. Palynol. *65*, 217-228.

GUY-OHLSON, D. (1992): *Botryococcus* as an aid in the interpretation of palaeoenvironment and depositional processes. - Rev. Palaeobot. Palynol. *71*, 1-15.

GUY-OHLSON, D. (1996): The Significance of Specific Microalgae in the Palaeoenvironmental Interpretation and Biostratigraphical Correlation of the Jurassic in Sweden. - Geo Research Forum *1-2*, 249-254.

GUY-OHLSON, D. and LINDQVIST, B. (1990): Palynomorph content of the uraniferous alum shales of Cambrian-Ordovician age in Sweden. - Ore Geology Rev. *5*, 525-536.

GUY-OHLSON, D. and LINDSTRÖM, S. (1994): Palaeoecology of the Early Permian strata at Heimefrontfjella, Dronning Maud Land, Antarctica. - Antarctic Science *6*, 507-515.

GUY-OHLSON, D. and NORLING, E. (1994): Jurassic sequences in Sweden. - Geobios *17*, 275-286.

HAJÓS, M. (1976): A pulai Put-3 számú fúrás felső pannóniai képződményeinek Diatóma flórája. - M.Á.F.I. évi jelentése az 1974 évről, 263-277.

HAJÓS, M. (1990): Diatoms in Alginite from Hungarian Pliocene crater lakes. In: 10th Diatom-Symposium 1988, Koelnidstein, 365-371.

HARRIS, T.M. (1938): The British Rhaetic flora. - British Museum (Nat. Hist.), 9-38.

HETÉNYI, M. (1979): Thermal degradation of the oil shale kerogen of Pula (Hungary) at 473 and 573 K. - Acta Miner.-Petr. Szeged. *24*, 99-111.

HETÉNYI, M. (1980): Thermal degradation of the organic matter of oil shale of Pula (Hungary) at 573-773 K. Acta Miner.-Petr. Szeged. *24*, 301-314.

HETÉNYI, M. (1985): Organic geochemical features of the maar-type oil shales of Hungary. - Acta Miner.-Petr. Szeged. *27*, 145-152.

HETÉNYI, M. (1987-1988): Methods for measuring the maturity of organic matter in diagenesis stage. - Acta Miner.-Petr. Szeged. *29*, 107-118.

HETÉNYI, M., MAITZ, K. and TÓTH, E. (1977): Contributions to the knowledge of the Hungarian oil shale kerogen I. Preliminary report on the results of the pyrolysis and selective oxidation. - Acta Miner.-Petr. Szeged. *23*, 165-175.

HETÉNYI, M. and PÁPAY, L. (1986): Type and evolution stage of Hungarian oil shale kerogen. - Acta Miner.-Petr. Szeged. *28*, 109-116.

HETÉNYI, M. and SIROKMÁN, K. (1978): Structural informations on the kerogen of the Hungarian oil shale. - Acta Miner.-Petr. Szeged. *23*, 211-222.
HETÉNYI, M. and VARSÁNYI, I. (1976): Contributions to the isolation of the kerogen in Hungarian oil shales. - Acta Miner.-Petr. Szeged. *22*, 231-239.
JARZEN, D.M. (1978): Some Maestrichtian palynomorphs and their phytogeographical and paleoecological implications. - Paleoecology 2, 29-38.
JÁMBOR, Á. (1980): A Dunántúli-középhegység pannóniai képződményei. Pulai alginit tagozat. - M.Á.F.I. évkönyve *62*, 125-129.
JÁMBOR, Á. and SOLTI, G. (1975): Geological conditions of the Upper Pannonian oil-shale deposits recovered in the Balaton Highland and Kemeneshát. - Acta Miner-Petr. Szeged. *22*, 9-28.
JÁMBOR, Á. and SOLTI, G. (1976): Geological conditions of the Upper Pannonian oil shale deposits recovered in the Balaton Highland and at Kemeneshát (Transdanubia, Hungary). - Annual Report of the Hungarian Geol. Inst. of 1974, 193-220.
KADOURI, A., DERENNE, S., LARGEAU, C., CASADEVALL, E. and BERKALOFF, C. (1988): Resistant biopolymer in the outer walls of *Botryococcus braunii*, B race. - Phytochemistry *27*, 551-557.
KEDVES, M. (1983): Étude paléobotanique sur les schistes pétrolifères du Tertiaire supérieur de Hongrie. - Revue de Micropaléontologie 26, 48-53.
KEDVES, M. (1986a): Dégradation expérimentale des colonies du genre *Botryococcus* des schistes pétrolifères du Tertiaire supérieur de Hongrie. - Acta Biol. Szeged. *32*, 39-48.
KEDVES, M. (1986b): Komplex (LM; TEM és vékonyréteg kromatográfiás) vizsgálatok olajpala növényi mikrofossziliáin. A complex study of plant microfossils of oil shale by LM, TEM and thin layer chromatography. - Bot. Közlem. *73*, 26-32.
KEDVES, M. (1987): Degradation of the sporoderm under natural and in vitro conditions. - XIV Internat. Bot. Congr. Berlin (West), Germany, Abstracts, 295.
KEDVES, M. (1988): Degradation of the sporoderm under natural and in vitro conditions. - Acta Biol. Szeged. *34*, 59-69.
KEDVES, M. (1991): Szerves növényi mikrofossziliák biopolimer organizációja. The biopolymer organization of the organic plant microfossils. - Discussiones Palaeontologicae *36-37*, 77-89.
KEDVES, M., ROJIK, I. and VÉR, A. (1991): Biopolymer organization of the partially degraded oil shale with the fragmentation method. - Plant Cell Biology and Development (Szeged) *1*, 28-31.
KEDVES, M., ROJIK, I. and VÉR, A. (1992): Ultrastructure and biopolymer organization of the *Botryococcus* colonies from Hungarian Alginite. - Workshop on Pyrolysis in Organic Geochemistry, Internat. Workshop, Szeged, Hungary, Abstract, 21,22.
KEDVES, M., TÓTH, A. and FARKAS, E. (1993): An experimental investigation of the biopolymer organization of both recent and fossil sporoderm. - Grana Suppl. *1*, 40-48.
KEDVES, M., TÓTH, A. and VÉR, A. (1993): Radial fivefold rotation: A new method in the study of the biopolymer organization of the sporoderm. - XV. Int. Bot. Congr. Yokohama, Japan, Abstracts, 28.
KEDVES, M., TÓTH, A. and VÉR, A. (1995): Radial fivefold rotation: A new method in the study of the biopolymer organization of the sporoderm. - Plant Cell Biology and Development (Szeged) *6*, 44-59.
KEDVES, M., TRIPATHI, S.K.M., VÉR, A., PÁRDUTZ, Á. and ROJIK, I. (1998): Experimental studies on *Botryococcus* colonies from Hungarian Upper Tertiary oil shale. - Plant Cell Biology and Development (Szeged) 9, 43-63.
KISS, I. (1939): Die Mikrovegetation der Natronwässer des Comit. Békés. - Folia Cryptogamica *4*, 217-266.
KOSANKE, R.M. (1951): A type of boghead coal from Illinois. - Amer. J. Sci. 249, 444-450.
KOSANKE, R.M. and MYERS, D.A. (1986): Palynological analysis of several Pennsylvanian coal beds from Santa Fe County, New Mexico. - New Mexico Geology, August, 54-56.
KRIVÁN-HUTTER, E. (1963): Microplancton from the Palaeogene of the Dorog Basin. I. Ann. Univ. Sci. Budapest de Rolando Eötvös nominatae, Sect. Geol. *6*, 71-91.
KVAČEK, Z., HABLY, L. and SZAKMÁNY, GY. (1994): Additions to the Pliocene flora of Gérce (Western Hungary). - Földt. Közlöny *124*, 69-87.
LARGEAU, C., CASADEVALL, E., KADOURI, A. and METZGER, P. (1984): Formation of *Botryococcus*-derived kerogens - Comparative study of immature torbanites and of the extant alga *Botryococcus braunii*. - Organic Geochemistry *6*, 327-332.
LARGEAU, C., DERENNE, S., CASADEVALL, E., BERKALOFF, C., COROLLEUR, M., LUGARDON, B., RAYNAUD, J.F. and CONNAN, J. (1990): Occurrence and origin of "ultralaminar" structures in "amorphous" kerogens of various source rocks and oil shales. - Org. Geochem. *16*, 889-895.
LARGEAU, C., DERENNE S., CLAIRAY C., CASADEVALL E., RAYNAUD J. F., LUGARDON B., BERKALOFF C., COROLLEUR M. and ROUSSEAU B. (1990): Characterization of various kerogens by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Morphological relationship with resistant outer walls in extant microorganisms. - Meded. Rijks. Geol. Dienst. *45*, 91-101.

LUGARDON, B., RAYNAUD, J.-F. et HUSSON, P. (1991): Données ultrastructurales sur la matière organique des kérogènes. - Palynosciences *1*, 69-88.
MEZŐSI, J. (1976): Data on the geology and mineralogy of the oil shale occurrence at Pula, Hungary. - Acta Miner.-Petr. Szeged. *22*, 195-200.
NAGY, E. (1965): Sur le role des organismes planctoniques du Neogene de la Montagne Mecsek, comme indicateurs de facies. - M.Á.F.I. évi jelentése az 1963 évről, 69-76. (en hongrois, résumé en français et en russe).
NAGY, E. (1969): Palynological elaborations the Miocene layers of the Mecsek Mountains. - Ann. Inst. Geol. Publ. Hung. *52*, 235-249.
NAGY, E. (1975a): A dunántúli olajpala-kutató fúrások rétegsorának palinológiai vizsgálata. - M.Á.F.I. évi jelentése az 1974 évről, 247-261.
NAGY, E. (1975b): Palynological investigations of alginites in Hungary. - Journal of Palynology *14*, 94-100.
NAGY, E. (1976): A dunántúli olajpala-kutató fúrások rétegsorának palinológiai vizsgálata. - M.Á.F.I. évi jelentése az 1974 évről, 247-263.
NAGY, E. (1992): A comprehensive study of Neogene sporomorphs in Hungary. - Geol. Hung. ser. Palaeontologica *53*, 1-379.
NAGY, E. (1993): A palaeoenvironmental reconstruction of the Hungarian Neogene. - Ann. Ref. Hung. Geol. Surv. 1991, 263-274.
NAGY, E. (1997): A palynological study of the Transdanubian alginites. - Studia Naturalia *10*, Early Pliocene volcanic environment, flora and fauna from Transdanubia, West Hungary ed.: L. HABLY, 153-175.
NARAYANA RAO, S.R. and MISRA, S.S. (1949): An oil-bearing alga from the Palana Lignite (? Eocene) of Rajputana. - Curr. Sci. *18*, 380-381.
PANKOW, H. (1976): Algenflora der Ostsee II. Plankton. - VEB Gustav Fischer Verlag, Jena.
RAINE, J.I. (1987): Jurassic plant microfossils and macrofossils from Murihiku, Manganui Valley, Awakino District (North Island, New Zealand). - N.Z. Geol. Survey Record *20*, 127-138.
RAVASZ, CS. (1976): Petrographic examinations of oil shale at Pula and Gérce. - Annual Report of the Hungarian Geol. Inst. of 1974, 221-246.
ROGALSKA, M. (1976): Stratigaphy of the Lower and Middle Jurassic in the Polish Lowlands on the basis of spore and pollen analysis. - Inst. Geol. Prace *78*,1-61 (Polish, Russian, English).
SOLTI, G. (1981): A pulai gejzirit. - M.Á.F.I. évi jelentése az 1979 évről, 241-247.
TAKAHASHI, M. (1993): Exine initiation and substructure in pollen of *Caesalpinia japonica* (*Leguminosae: Caesalpinioideae*). - Amer. J. Bot. *80*, 192-197.
TEGELAAR, E.W., DE LEEUW, J.W., DERENNE, S. and LARGEAU, C. (1989): A reappraisal of kerogen formation. - Geochimica et Cosmochimica Acta *53*, 3103-3106.
TEGELAAR, E.W., DE LEEUW, J.W. and SAIZ-JIMENEZ, C. (1989): Possible origin of aliphatic moieties in humic substances. - Sci. tot. Envir. *81/82*, 1-17.
TEMPLIER, J., DIESENDORF, C., LARGEAU, C. and CASADEVALL, E. (1992): Metabolism of n-alkadienes in the A race of *Botryococcus braunii*. - Phytochemistry *31*, 113-120.
TEMPLIER, J., LARGEAU, C., CASADEVALL, E. and BERKALOFF, C. (1992): Chemical inhibition of resistant biopolymers in outer walls of the A and B races of *Botryococcus braunii*. - Phytochemistry *31*, 4097-4104.
TRAVERSE, A. (1955): Pollen analysis of the Brandon lignite of Vermont. - Bur. Min. Rept. Repts. Invest. *5151*, 1-107.
VÉR, A. (1994): LM investigations of different stained fossil *Botryococcus* colonies. - Plant Cell Biology and Development (Szeged) *5*, 11-19.
VOLKHEIMER, W., CACCAVARI DE FILICE, M.A. y SEPULVEDA, E. (1977): Datos palinologicos de la Formation Ortiz (Grupo La Anaga), Cretacico inferior de La Cuenca Neuquina (Republica Argentina). - Ameghiniana *14*, 59-74.
WICANDER, R., FOSTER, C.B. and REED, J.D. (1996): Chapter 7 *Green* and *Blue-Green* Algae, 7E-*Gloeocapsomorpha*. In: Palynology, principles and applications eds.: JANSONIUS, J. and MCGREGOR, D.C. - American Association of Stratigraphic Palynologists Foundation *1*, 215-225.
WILLE, N. (1910): *Botryococcaceae*. In: ENGLER und PRANTL: Die natürlichen Pflanzenfamilien. Lief. 32-35, Wilhelm Engelmann, Leipzig, 32-35.
WODEHOUSE, R.P. (1933): The oil shale of the Eöcene Green River Formation. - Bull. Torr. Bot. Club. 60, 479-524.
WOLF, F.R. and COX, E.R. (1981): Ultrastructure of active and resting colonies of *Botryococcus braunii (Chlorophyceae)*. - J. Phycol. *17*, 395-405.

6. CHLOROPLAST THYLAKOID MEMBRANES IN THE INTINE OF RAGWEED POLLEN GRAINS AS A POSSIBLE CONSTITUENT IN THE BIOSYNTHESIS OF ITS EXTREME ALLERGENIC ANTIGENS

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Abstract

During our experimental Transmission Electron Microscope (TEM) investigations on the partially dissolved ragweed pollen grains chloroplasts were found in the intine. Till this time chloroplasts or other kind of plastids were not observed in the pollen wall. On the surface of the thylakoid membranes different kinds of molecular organizations were observed which may be important in the biosynthesis of antigens. But as an alternative hypothesis it must be mentioned, that the products of assimilation are factors of extreme allergenic character of ragweed pollen grains. The accumulation of extreme allergenic antigens, mainly antigen E in the wall (intine) of ragweed pollen grains have been described by several authors. Direct contact come with the intine of the pollen grains and the mucosa of the human respiratory organs. In consequence of the body temperature and the moist surface of the respiratory passages pollen tube development begins and the antigens enter the human cells.

Key words: Ragweed pollen, experimental ultrastructure, allergenic antigens.

Introduction

The extreme allergenic character of the antigens of ragweed pollen grains was pointed out, in the first place, in aeropalynological papers. GRATER and STEMEN (1967) emphasized, that among the numerous pollen forms the antigenic agents of ragweed have been the most intensive studied. Based on the paper of O'ROURKE (1996), *Ambrosia*, with 45 species is the most important aeroallergen in North America. Two species (*Ambrosia trifida*, and *A. elatior*) have 52 identifiable antigens. Of these, two are acidic proteins and major antigens (E and K). The pollen wall (intine) localization of antigenic and allergenic proteins in the ragweed pollen grains was established also by several authors (KNOX and HESLOP-HARRISON, 1970, 1971, KNOX, HESLOP-HARRISON and REED, 1970, KNOX, WILLING and ASHFORD, 1972). TEM results of *Ambrosia* pollen grains were published by PAYNE and SKVARLA (1970), but acetolysed material was used, so the intine and the protoplasm were degraded, and the ultrastructure of the ectexine was described.

During our ultrastructure studies on partially dissolved and degraded pollen grains, we established in the partially dissolved pollen grains of *Platanus hybrida* with 50% glycerine, that the organelles were in an excellent preservation (KEDVES, PÁRDUTZ and

TÓTH, 1999). We have started several experiment on fresh ragweed pollen grains and the results of the partially, dissolved pollen grains are the most surprising.

Materials and Methods

Fresh ragweed pollen grains were dissolved in 50% glycerine at 30 °C, for 30 days the washed material was postfixed in OsO_4 embedded in Araldite. The investigations were made on a Tesla BS-540 (resolution 6-7 Å) and Opton EM-902 (resolution 2-3 Å) instrument.

Results and Discussion

At the general survey picture (Plate 6.1.) the characteristic *Compositae* type outer wall ectexine, (ect) with spinae (sp), on the apertural area (aa), the oncus (on), the endexine (en), the intine (in) with some chloroplasts (chl) are well shown. The organelles of the protoplasm are also well illustrated, the characteristic plasma membrane (pm) may be emphasized. The chloroplast of the intine is connected with the plasma membrane (Plate 6.1., plate 6.2., figs. 1,2). The highly magnified pictures of the chloroplasts were taken with Opton instrument, illustrate the characteristic thylakoid membranes (Plate 6.2., figs. 1,2) with the molecular organization (Plate 6.3.). There are granules of different size on the membranes.

This organization, together with chemistry and function, were described in detail in the cell biology or molecular cell biology handbooks, (cf. AVERS, 1986). The freze-fractured picture from the thylakoid membranes of STAEHELIN, CARTER and MCDONNEL, was published in 1980. Four different membrane faces were distinguished with the superficial molecular systems. The function of these was described by BOGORAD (1981); e.g., ADP + P $\rightarrow$ ATP, and further functions were established. From the point of view of the allergenic character of ragweed pollen grains, it is important, that the place of the above mentioned functions is the intine, which is also the place of the accumulation of the antigens.

Plate 6.1.

Ambrosia artemisiifolia L. General survey TEM picture from the partially dissolved pollen grains. The picture was taken with an instrument of 6-7 Å of resolution. Experiment No: 1045. Negative no: 7542, 9.000x.
Abbreviations: aa = apertural area, on = oncus, sp = spinae, ect = ectexine, en = endexine, in = intine, chl = chloroplast, pm = plasma membrane.

Plate 6.2.

1,2. *Ambrosia artemisiifolia* L. TEM pictures from the chloroplast from the intine were taken with an instrument of 2-3 Å. Detail from the chloroplast, the plasma membrane and the protoplasm. 1. Negative no: 7865, 80.000x, 2. Negative no: 7866, 150.000x.

Plate 6.3.

Ambrosia artemisiifolia L. Detail of the thylakoid membranes. The electron dense granular system, which may be identified as the superficial molecular structures of STAEHELIN, CARTER and MCDONNEL 1980, after AVERS (1986, p. 388) are well shown. Negative no: 7867, 400.000x.

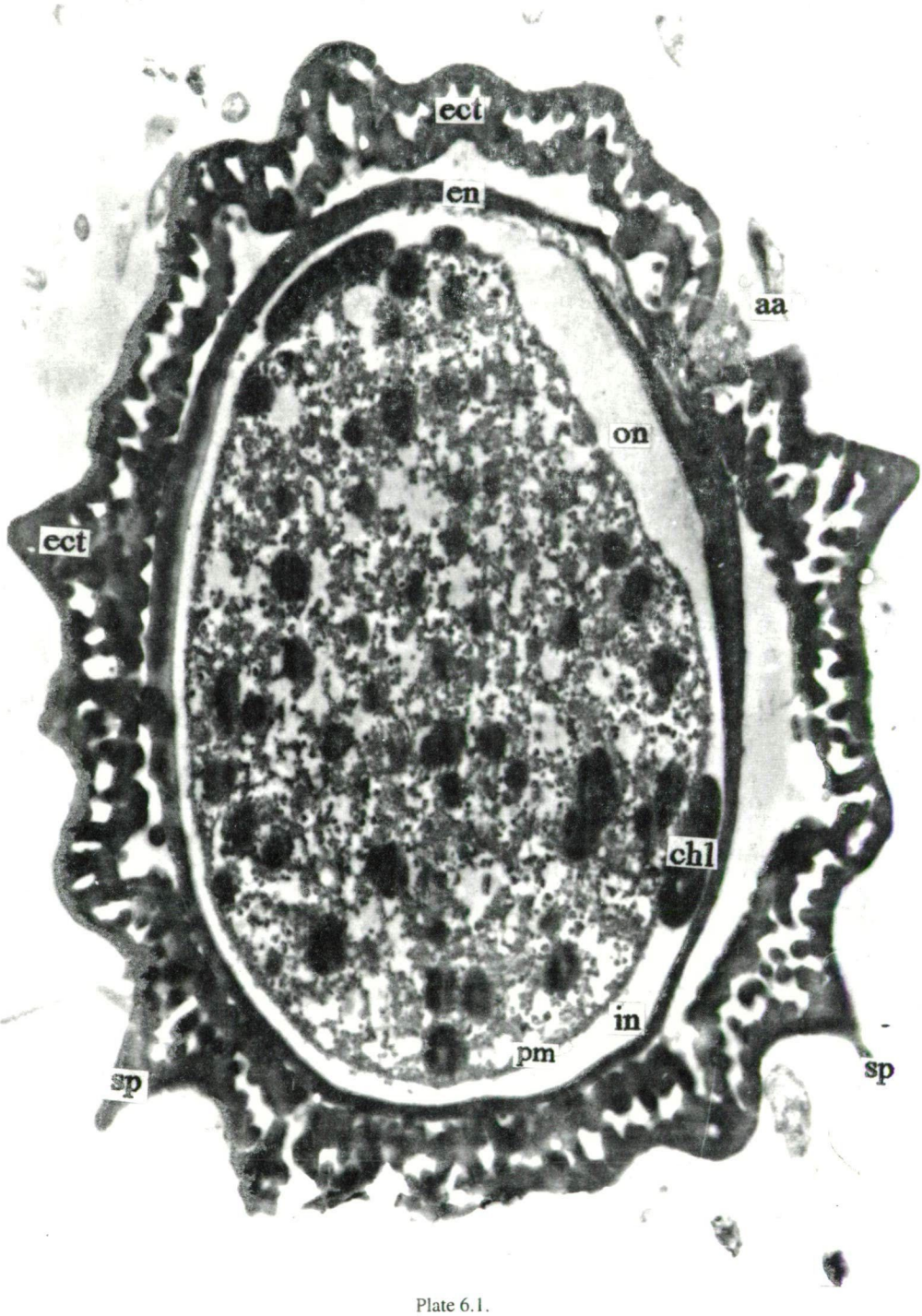

Plate 6.1.

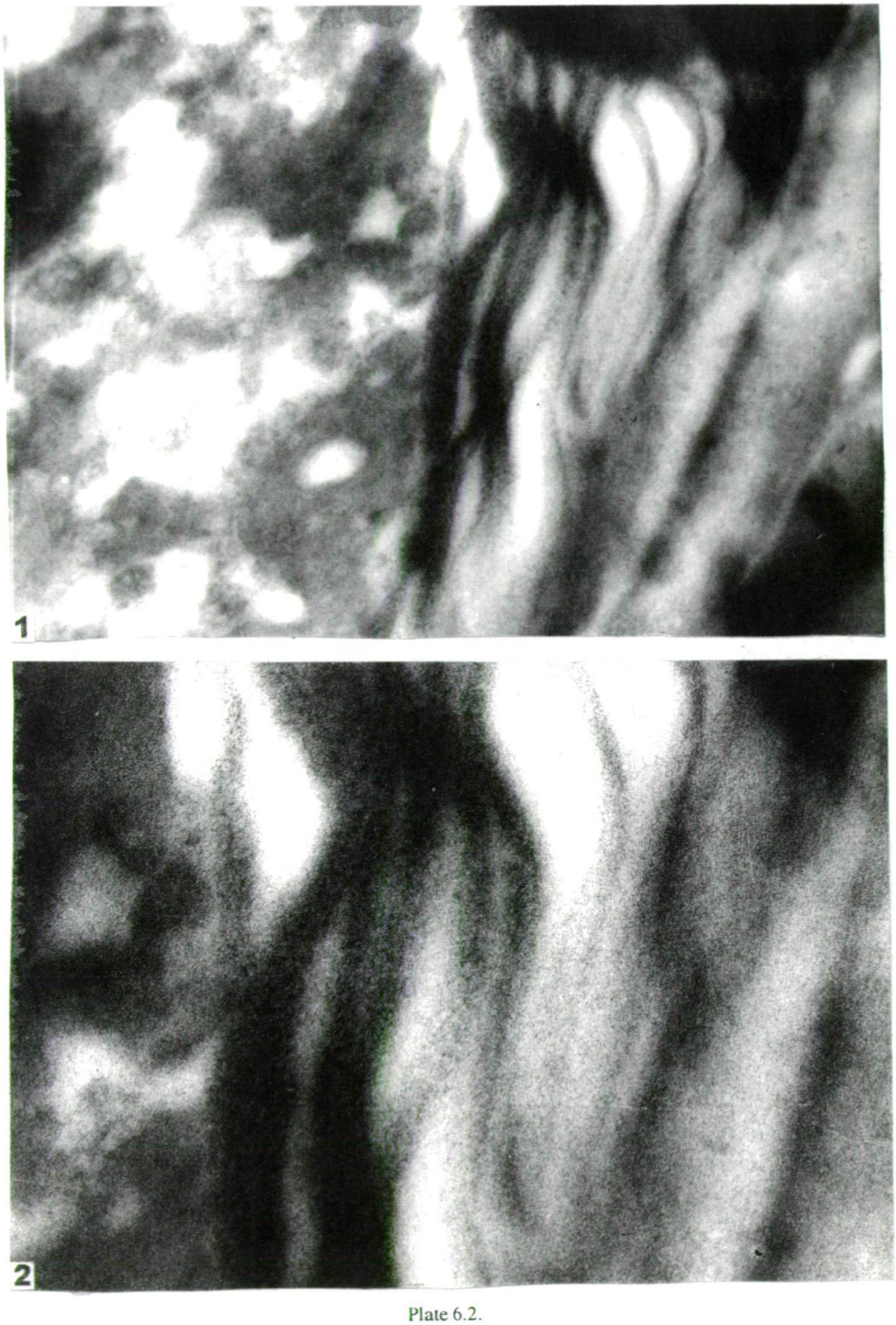

Plate 6.2.

Plate 6.3.

Conclusions

Taking into consideration the complex function of the thylakoid membranes it may be presumed, that these are in connection to the biosynthesis of the peculiar antigens of ragweed pollen grains. In addition we should like to point out that the products of the chloroplasts in the intine are factors of the reinforcement effect of the antigens. Finally, it is hoped that the experimental palynological method will be useful to better understand the allergenic effect of the pollen grains.

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References

AVERS, C.J. (1986): Molecular Cell Biology. - B. Cummings P.C. U.S.A.

GRATER, W.C. and STEMEN, T.R. (1967): The plant, the pollen and the patient. - Rev. Palaeobot. Palynol. *4*, 187-192.

KEDVES, M., PÁRDUTZ, Á. and TÓTH, A. (1999): Ultrastructure of partially dissolved pollen grains of *Platanus hybrida* BROT. - Plant Cell Biology and Development (Szeged) *10*, 83-90.

KNOX, R.B. and HESLOP-HARRISON, J. (1970): Pollen-wall proteins: localisation and enzymatic activity. - J. Cell Sci. *6*, 1-27.

KNOX, R.B. and HESLOP-HARRISON, J. (1971): Pollen-wall proteins: localisation of antigenic and allergenic proteins in the pollen grain walls of *Ambrosia* spp. (ragweeds). - Cytobios *4*, 49-54.

KNOX, R.B., HESLOP-HARRISON, J. and REED, C. (1970): Localisation of antigens associated with the pollen wall by immunofluorescence. - Nature *225*, 1066-1068.

KNOX, R.B., WILLING, R.R. and ASHFORD, A.E. (1972): Role of Pollen-Wall Proteins as Recognition Substances in Interspecific Incompatibility in Poplars. - Nature *237*, 381-383.

O'ROURKE, M.K. (1996): New Frontiers in Palynology 23F - Medical Palynology. In: Palynology, principles and applications (eds: JANSONIUS, J. and MCGREGOR, D.C.). - American Association of Stratigraphic palynologists Foundation, Publishers Press, Salt Lake City, Utah, *3*, 945-955.

PAYNE, W.W. and SKVARLA, J.J. (1970): Electron microscope study of *Ambrosia* pollen (*Compositae: Ambrosieae*). - Grana *10*, 89-100.

7. TRANSMISSION ELECTRON MICROSCOPIC STUDIES ON PARTIALLY DEGRADED POLLEN GRAINS OF PHOENIX SYLVESTRIS LINN.

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Abstract

Pollen grains of *Phoenix sylvestris* L. were partially degraded with the help of 2-aminoethanol and potassium permanganate and were investigated with the TEM. Six stages of degradation in pollen wall were studied. Linear and cyclic molecules arranged in pentagonal and hexagonal symmetry were observed in the degraded ectexine. On the tectal surface more or less radially oriented linear molecules were observed. The strongest degradation was achieved after treating the pollen with 2-aminoethanol for 72 hours and with aqueous diluted $KMnO_4$ for 24 hours.

Key words: Palynology, *Phoenix sylvestris*, experimental TEM.

Introduction

Extant pollen of *Phoenix sylvestris* L. from India were investigated to observe the molecular structure in partially degraded exine. Significance of these studies lies in observing the resistance of fossil pollen exines at the time of diagenesis. Pollen grains having affinity with those of recent palms are profusely recorded in Tertiary sediments over the world. Experimental studies on recent palm pollen of *Phoenix sylvestris* were carried out to note the changes in exine at molecular level as a result of treatment with 2-aminoethanol. Recent experiments have shown that changes in exine molecules largely depend upon the resistance of pollen walls which varies considerably from species to species (KEDVES and GÁSPÁR, 1994a,b, KEDVES et al., 1998, etc.). Also, the resistance of different layers of exine is variable and differential degradation can be achieved by using chemicals at various stages (FAEGRI, 1956, SOUTHWORTH, 1974, 1985a,b, 1986a,b, ROWLEY, 1978, 1990, 1995, ROWLEY and PRIJANTO, 1977, AUDRAN, 1981, ABADIE, HIDEUX and ROWLEY, 1986-1987).

Present study was undertaken as a collaborative research under the Exchange of Scientist Programme between Hungarian Academy of Sciences, Budapest and Indian National Science Academy, New Delhi.

Materials and Methods

The polleniferous material for investigation was collected from plant growing in natural habitat in eastern Uttar Pradesh, India. For each experiment 5 mg pollen-material was used. Details of the experiments are as follows:

1/7 - 1314 1 ml 2-aminoethanol, duration 24 hours
1/7 - 1315 1 ml 2-aminoethanol, duration 48 hours
1/7 - 1316 1 ml 2-aminoethanol, duration 72 hours
1/7 - 1317 1 ml 2-aminoethanol, duration 24 hours
10 ml 0.01 M $KMnO_4$, aq. dil., duration 24 hours
1/7 - 1318 1 ml 2-aminoethanol, duration 48 hours
10 ml 0.01 M $KMnO_4$, aq. dil., duration 24 hours
1/7 - 1319 1 ml 2-aminoethanol, duration 72 hours
10 ml 0.01 M $KMnO_4$, aq. dil., duration 24 hours

Temperature for all experiments was 30 °C.

For TEM investigations the degraded pollen grains were fixed with OsO_4 aq. dil., dehydrated and embedded in Araldite (Durcupan, Fluka). The ultrathin sections were made on Porter Blum ultramicrotome in the Electron Microscopy Laboratory of the Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged. The microphotographs were taken on Opton EM - 902 (resolution 2-3 Å) and on Tesla BS - 540 (resolution 6-7 Å).

Results

Experiment No. 1/7 - 1314 (Plate 7.1., plate 7.2., figs. 1,2)

Degradation of protoplasm started. Protoplasmic exudation through the aperture is observed. Electron dense granular areas are clearly discernible in the protoplasmic contents. Characteristic light zone, probably at the place of intine, noticeable between the degraded ectexine and the protoplasm. Degradation of ectexine also started (Plate 7.2., fig. 2).

Experiment No. 1/7 - 1315 (Plate 7.2., figs. 3,4)

Degradation of protoplasm, as observed after the previous experiment, is noticed. Ectexine degradation is a little advanced. Sometimes fine granular structures are in the tectum (Plate 7.2., fig. 4).

Experiment No. 1/7 - 1316 (Plate 7.3., figs. 1-3., plate 7.4., fig. 1)

Protoplasm is completely destroyed and partial degradation of ectexine is noticed. The degraded ectexine at this stage enables the study of different kinds of molecular arrangements in the sporopollenin. Linear and cyclic molecules arranged in pentagonal or hexagonal symmetry are clearly observed (Plate 7.3., fig. 3).

On tectal surface more or less radially oriented linear molecules are observed (Plate 7.3., fig. 2). In Angstrom dimension several kinds of biopolymer units are also observed. Regular pentagonal unit in Angstrom dimension is observed (Plate 7.4., fig. 1).

Experiment No. 1/7 - 1317 (Plate 7.4., figs. 2-4, plate 7.5.)

The molecular structure and the larger biopolymer units are seen. Large number of cyclic structures are noticed at the tectal surface (Plate 7.4., fig. 3). Different kinds of cyclic molecules and cluster of molecules are also seen (Plate 7.5). Biopolymer structures in Angstrom dimension are not observed. Disintegration of molecular system is observed in some parts.

Experiment No. 1/7 - 1318 (not illustrated)

Results of this experiment are identical to those of the previous experiment.

Experiment No. 1/7 - 1319 (Plate 7.6., figs. 1-3)

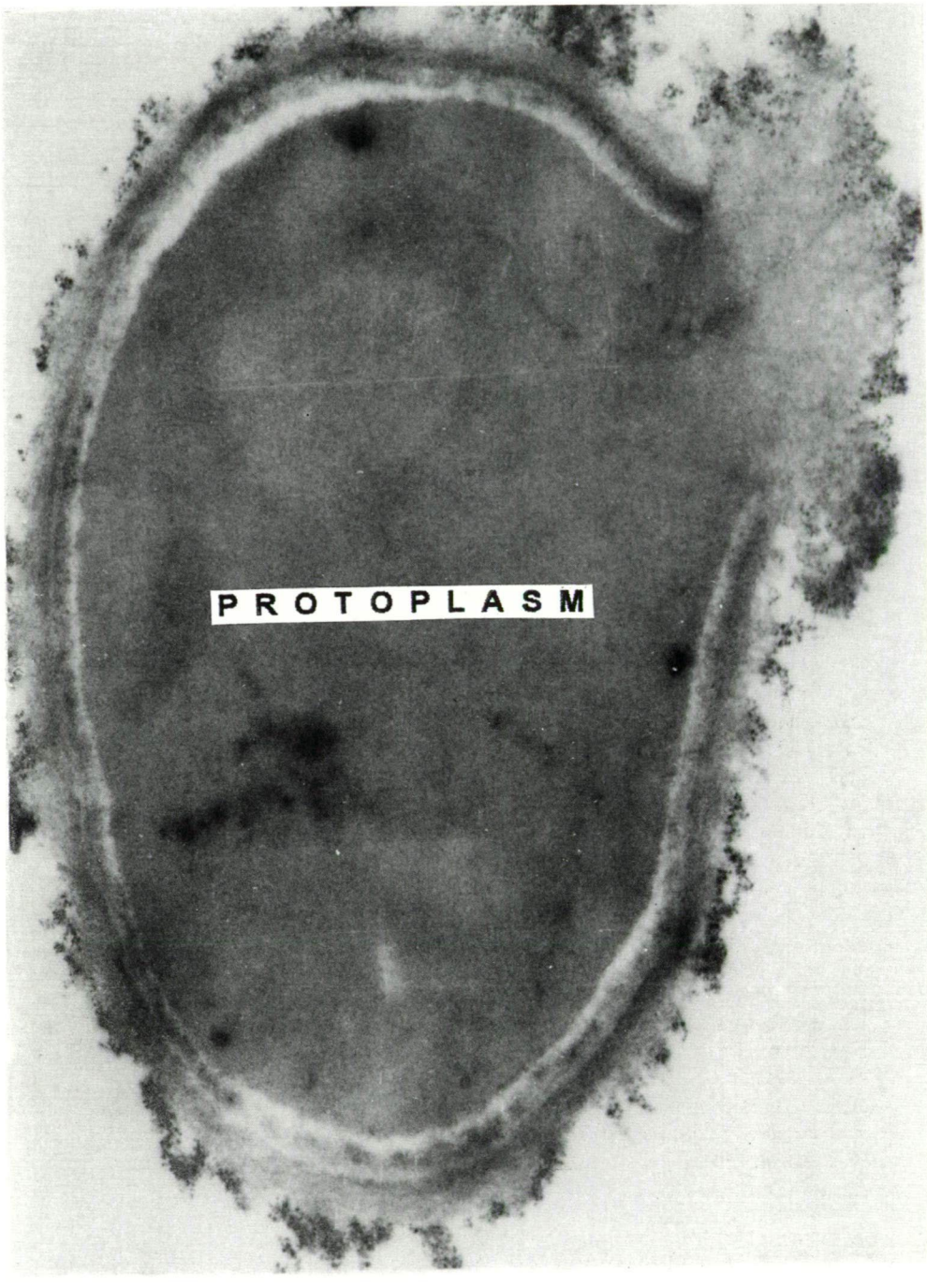
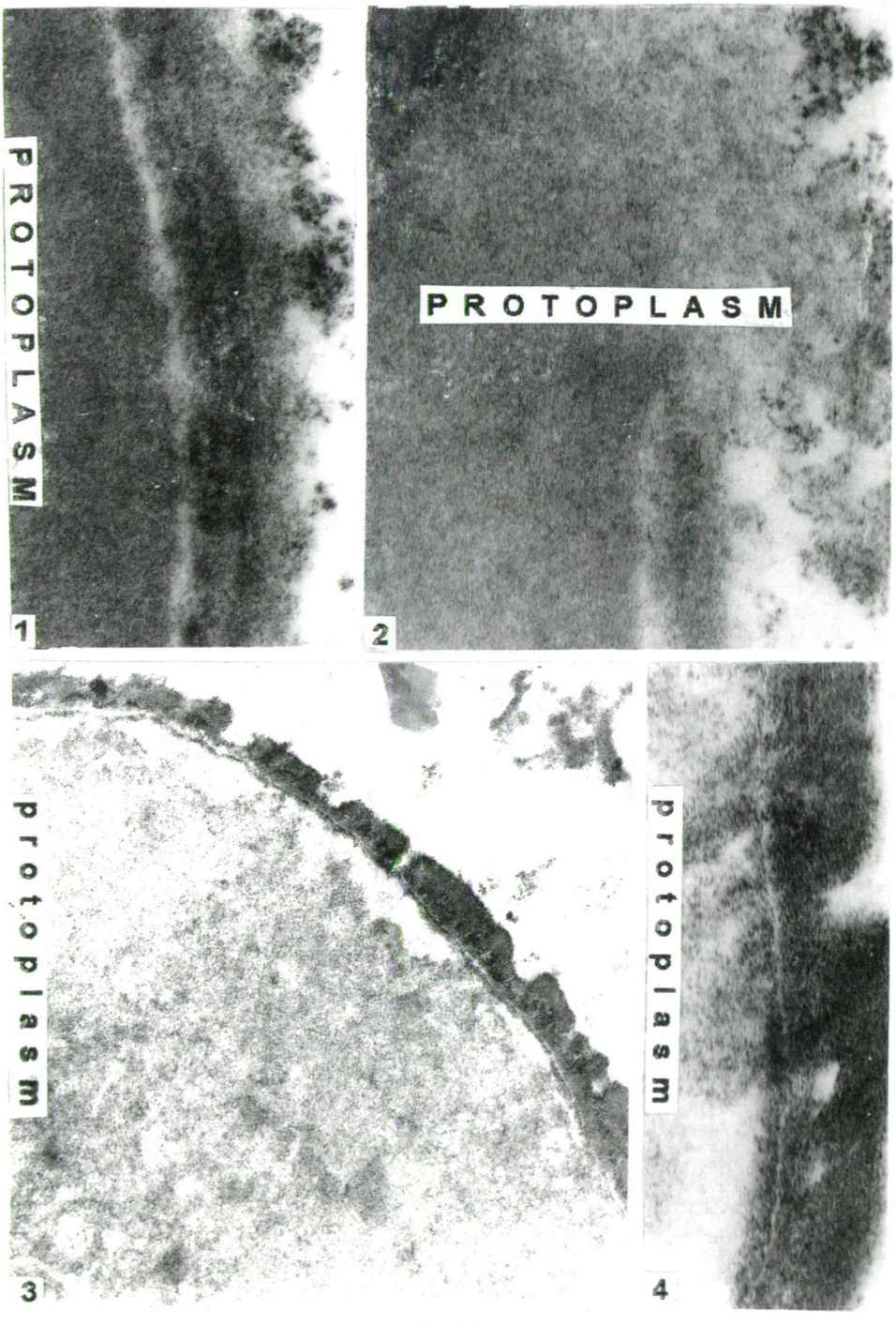


Plate 7.1.



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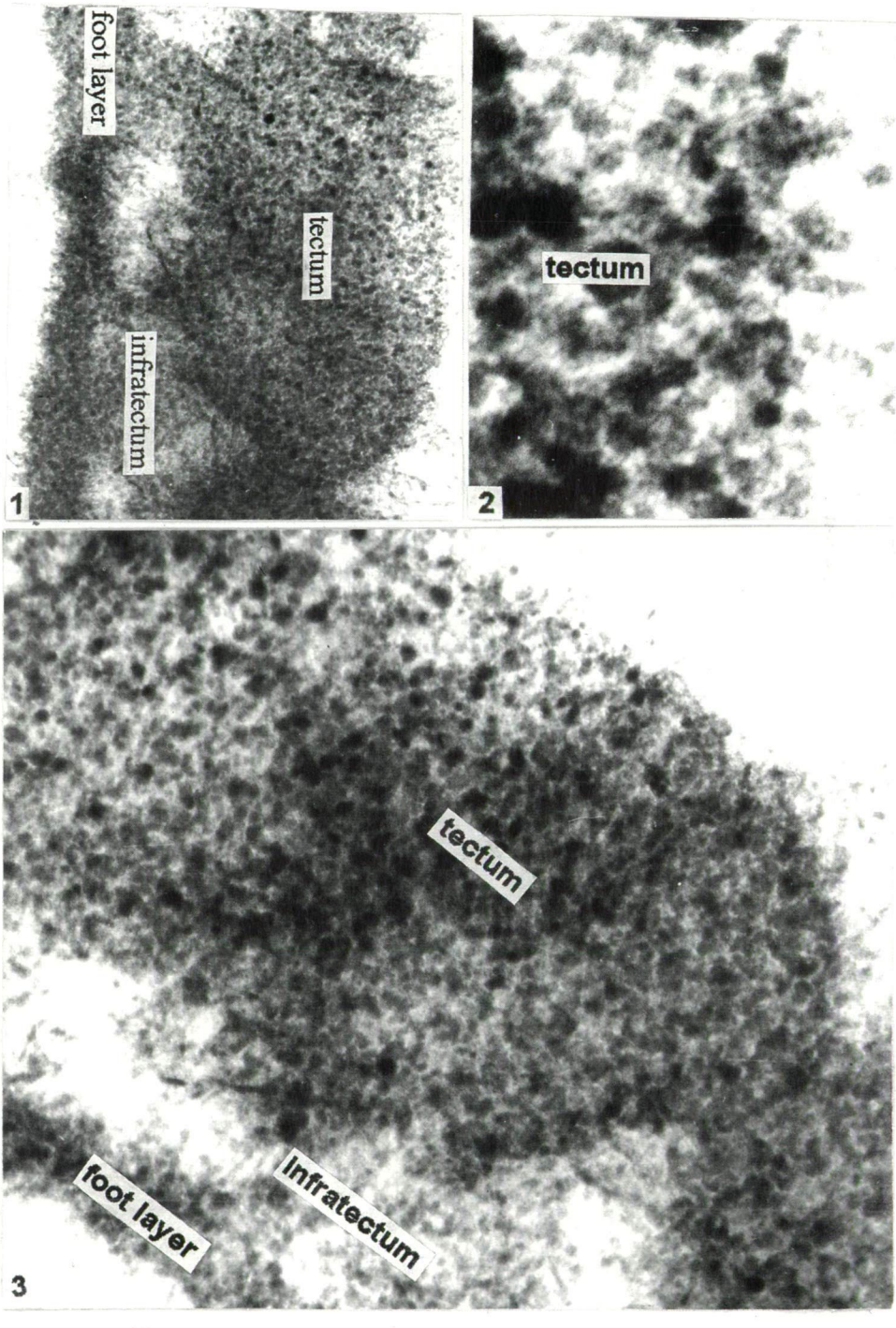
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Plate 7.3.

The strongest degradation is observed. Several linear molecular structures were also noticed (Plate 7.6., fig. 3) which may be part of the stabilizing molecular system of the metastable quasi-crystalloid skeleton. Remnants of the biopolymer skeleton in Angstrom dimension is also seen (Plate 7.6., fig. 2).

Discussion and Conclusions

Degradation of exine with the help of 2-aminoethanol has resulted into observation of substructures below the surface of sporopollenin (SOUTHWORTH, 1974, ROWLEY, 1995, ROWLEY et al., 1981). These substructures are radially oriented and are disposed at right angles to the lamellations of the endexine in *gymnosperms* and *angiosperms* and exospores of *pteridophytic* spores (ROWLEY, 1995). Experimental studies have demonstrated two levels of degradative resistance in different components of the exine as a response to various chemical treatments. The polymerization level of sporopollenin may be one of the reasons for differential resistance between new and old components of exine. Exine of mature pollen when dissolved in 2-aminoethanol results into separation of dializable autofluorescent sporopolleninous component and non-dializable in situ filamentous or fibrillar fraction. With use of preferential staining methods the filamentous fraction, termed as glycocalyx elements, has been demonstrated to be composed of polysaccharides and proteins. The fibrillar elements of the pollen wall get encapsulated by the sporopollenin giving rise to characteristic exinal morphologies. Degradation of exine with 2-aminoethanol at low temperature enables the study of biopolymer structures and their arrangement as it does not completely erode the sporopolleninous component.

Plate 7.1.

Phoenix sylvestris L.
Experiment No. 1/7 - 1314. Ultrastructure microphotograph of the pollen grain. Negative no: 7056, 7.500x.

Plate 7.2.

Phoenix sylvestris L.
1,2. Experiment No. 1/7 - 1314.
1. Details of the partially degraded exine and protoplasm. Negative no: 7059, 50.000x.
2. Highly magnified part of exuding protoplasm. Negative no: 7057, 50.000x.
3,4. Experiment No. 1/7 - 1315.
3. Details of the ultrastructure of the pollen grains. Negative no: 7087, 15.000x.
4. Details of the ultrastructure of the ectexine. Negative no: 7085, 50.000x.

Plate 7.3.

Phoenix sylvestris L.
1-3. Experiment No. 1/7 - 1316.
1. Biopolymer units of the partially degraded ectexine. Negative no: 7096, 150.000x.
2. Superficial molecular structures of the tectum. Negative no: 7078, 1.000.000x.
3. Molecular structure of the ectexine. Highly magnified, showing details of fig. 1. Negative no: 7077, 250.000x.

During the present study biopolymers were observed in the ectexine when pollen grains were treated with 2-aminoethanol coupled with mild heating at 30 °C and without using potassium permanganate. In some experiments (1/7 - 1317 to 1/7 - 1319) dil. aqueous $KMnO_4$ was also used. Studies have demonstrated that the organization of the molecules is mostly cyclic but linear structures are also observed (Plate 7.3., fig. 3). These structures are arranged in pentagonal or hexagonal symmetries. Some elongated structures were also noticed which are possibly helical in nature (Plate 7.3., fig. 2).

Regular pentagonal molecules in Å dimension will enable the study of symmetry in biopolymer organization. Highly organised structures and clusters of molecules were observed (Plate 7.5). Results of the present study indicate that the sporopollenin in ectexine is comparatively resistant. The TEM studies document that ectexine of fossil and recent Palm is compact and homogeneous (THANIKAIMONI, 1970, HARLEY, 1990, FERGUSON and HARLEY, 1993, ZAVADA, 1983). Our study has confirmed these results.

Acknowledgements

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Plate 7.4.

Phoenix sylvestris L.

1. Experiment No. 1/7 - 1316. Regular pentagonal biopolymer unit. Negative no: 7083, 2.000.000x.

2-4. Experiment No. 1/7 - 1317.

2. Details of partially degraded ectexine. Negative no: 7084, 150.000x.
3. Superficial biopolymer structure. Negative no: 7089, 150.000x.
4. Details of partially degraded ectexine. Highly magnified part of figure 2. Negative no: 7085, 250.000x.

Plate 7.5.

Phoenix sylvestris L.

Experiment No. 1/7 - 1317. Molecular system of different organization levels of the partially degraded ectexine. Negative no: 7088, 2.000.000x.

Plate 7.6.

Phoenix sylvestris L.

1-3. Experiment No. 1/7 - 1319.

1. Ultrastructure and biopolymer system of the partially degraded ectexine. Negative no: 7097, 150.000x.
2. Biopolymer units of the partially degraded ectexine. Negative no: 7099, 1.000.000x.
3. Linear biopolymer units of the partially degraded ectexine. Negative no: 7093, 250.000x.

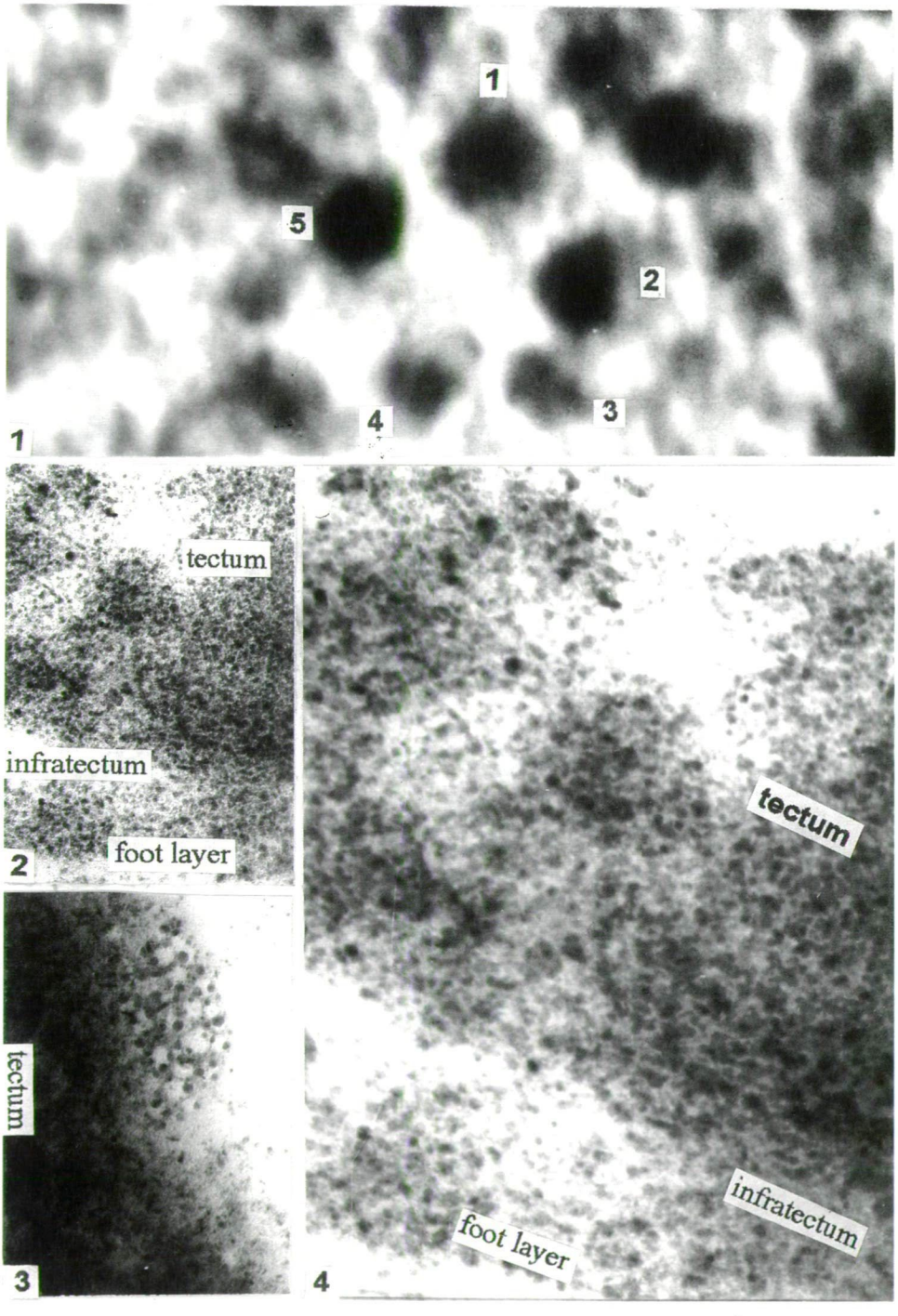
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Plate 7.4.

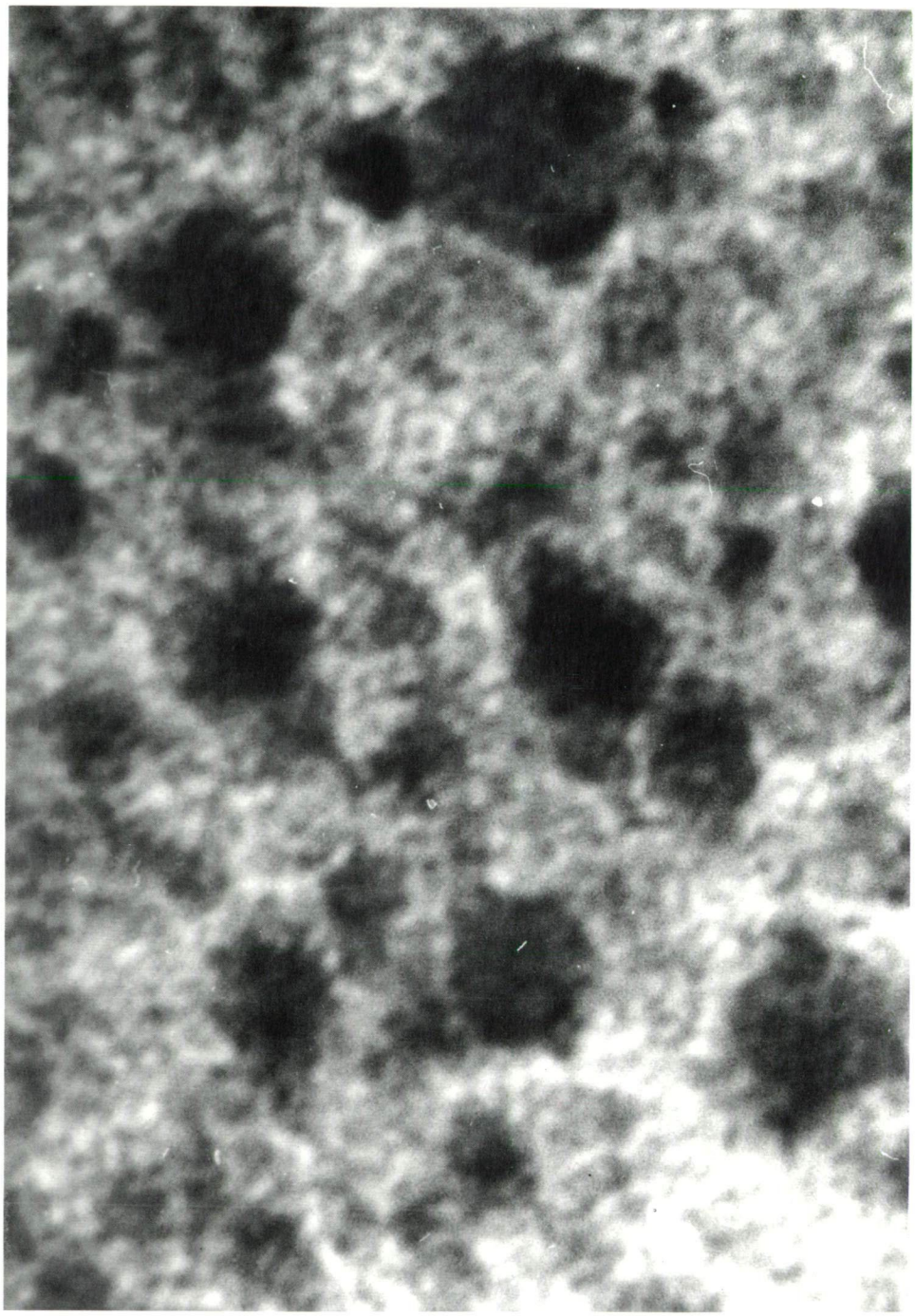

Plate 7.5.

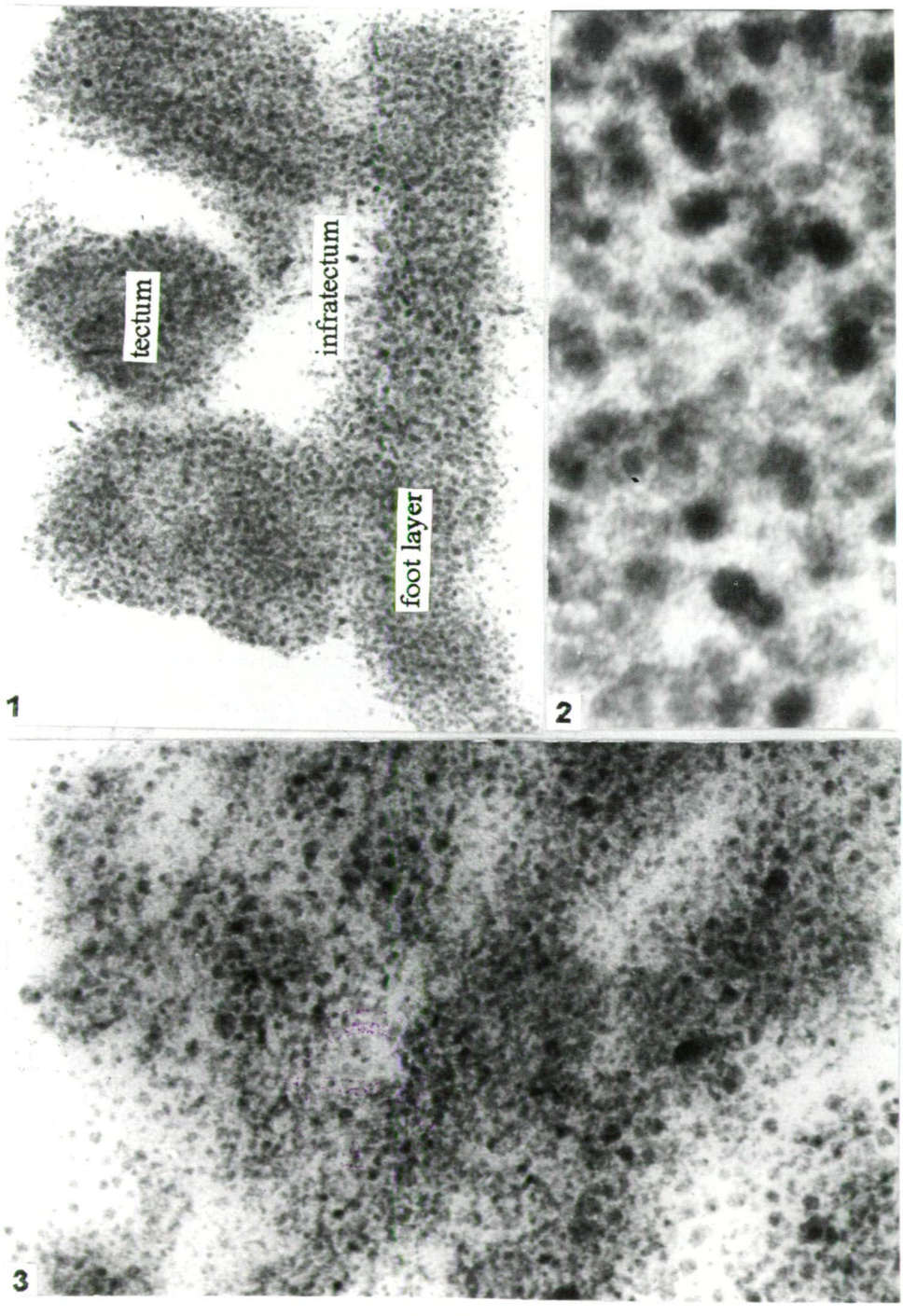

Plate 7.6.

References

ABADIE, M., HIDEUX, M. and ROWLEY, J.R. (1986-87): Ultrastructural cytology of the anther. II. Proposal for a model of exine considering a dynamic connection between cytoskeleton, glycolemma and sporopollenin - synthesis. - Ann. des Sci. Nat. Bot. Paris 13^{e} Ser. Tom. *8*, 1-16.

AUDRAN, J. C. (1981): Degeneration of *Trachymena pilosa* exine by osmium tetroxide used in impregnation technique. – Planta *152*, 282–284.

BROOKS, J. and SHAW, G. (1978): Sporopollenin: A review to its chemistry, palaeochemistry and geochemistry. - Grana *17*, 91-97.

FAEGRI, K. (1956): Recent trends in Palynology. – Bot. Rev. *22*, 639-664.

FERGUSON, I. K. and HARLEY, M.M. (1993): The significance of new and recent work on the morphology of the *Palmae*. - Kew Bull. *48*, 205-213.

HARLEY, M.M. (1990): Occurrence of simple tectate, monosulcate or trichotomosulcate pollen grains within the *Palmae*. - Rev. Palaeobot. Palynol. *64*, 137-147.

KEDVES, M. (1980): Morphological investigation of recent Palmae pollen grains. – Acta Bot. Acad. Sci. Hung. *26*, 339-373.

KEDVES, M. et GÁSPÁR, I. (1994a): Les altérations secondaires des spores et des grains de pollen dissous partiellement. – X Simposio de Palinologia A.P.L.E. Valencia, programa y resumenes, 53.

KEDVES, M. et GÁSPÁR, I. (1994b): Les altérations sécondaires des spores et des grains de pollen dissous partiellement. – Trabajos de Palinologia Básica y Aplicada. – X Simposio de Palinologia (A.P.L.E.), Univ. de Valencia, 153-161.

KEDVES, M., HORVÁTH, E., MÉSZÁROS, E., MÉSZÁROS, R., RONTÓ, G., SZLÁVIK, N., GAUDÉNYI, SZ. and KALMÁR, Á. (1998): LM investigations of partially dissolved sporomorphs II. – Plant Cell Biology and Development (Szeged) *9*, 76-87.

ROWLEY, J.R. (1978): The origin, ontogeny and evolution of the exine. - IV Int. Palynol. Conf. Lucknow (1976-77) *1*, 126-136.

ROWLEY, J. R. (1990): The fundamental structure of the pollen exine. – Pl. Syst. Evol. (Suppl. 5), 13-29.

ROWLEY, J.R. (1995): Are the endexines of *pteridophytes, gymnosperms* and *angiosperms* structurally equivalent? - Rev. Palaeobot. Palynol. *85*, 13-34.

ROWLEY, J.R., DAHL, A.O., SENGUPTA, S. and ROWLEY, J.S. (1981): A model of exine substructure based on dissection of pollen and spore exines - Palynology *5*, 107-152.

ROWLEY, J.R. and PRIJANTO, B. (1977): Selected destruction of the exine of pollen grains. - Geophytology *7*, 1-23.

SOUTHWORTH, D. (1974): Solubility of pollen exines. - Amer. J. Bot. *61*, 36-44.

SOUTHWORTH, D. (1985a): Pollen exine substructure. I. *Lilium longiflorum*. - Amer. J. Bot. *72*, 1274-1283.

SOUTHWORTH, D. (1985b): Pollen exine substructure. II. *Fagus sylvatica*. - Grana *24*, 161-166.

SOUTHWORTH, D. (1986a): Pollen exine substructure. *III. Juniperus communis*. – Can. J. Bot. *64*, 983-987.

SOUTHWORTH, D. (1986b): Substructural organization of pollen exines. – Pollen and Spores: Form and Function, 61-69.

THANIKAIMONI, G. (1970): Les *Palmiers*: Palynologie et Systématique. - Inst. Français de Pondichéry. - Trav. Sect. Sci. et Tech. *11*, 1-186.

ZAVADA, M.S. (1983): Comparative Morphology of *Monocot* Pollen and Evolutionary Trends of Apertures and Wall Structures. - The Bot. Rev. *49*, 331-379.

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